

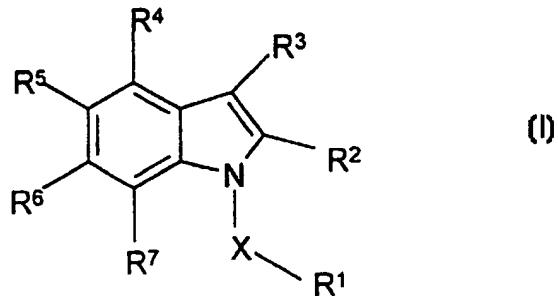
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(54) Title: ANTI-INFLAMMATORY INDOLE DERIVATIVES

(57) Abstract

Compounds of formula (I) or a pharmaceutically acceptable salt, *in vivo* hydrolysable ester, or amide thereof, where X is CH_2 or SO_2 ; R^1 is an optionally substituted aryl or heteroaryl ring; R^4 is a group OR^{15} or $\text{S}(\text{O})_q\text{R}^{15}$, where q is 0, 1 or 2 and R^{15} is a substituted hydrogen-containing alkyl group; and R^2 , R^3 , R^5 , R^6 and R^7 are specified organic groups. These compounds are useful in therapy, in particular of inflammatory disease, and methods of producing them as well as pharmaceutical compositions containing them are also described and claimed.



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ANTI-INFLAMMATORY INDOLE DERIVATIVES

The present invention relates to chemical compounds, to their production as well as to pharmaceutical compositions containing them and to their use in therapy, in particular of 5 inflammatory disease.

MCP-1 is a member of the chemokine family of pro-inflammatory cytokines which mediate leukocyte chemotaxis and activation. MCP-1 is a C-C chemokine which is one of the most potent and selective T-cell and monocyte chemoattractant and activating agents known. MCP-1 has been implicated in the pathophysiology of a large number of inflammatory 10 diseases including rheumatoid arthritis, glomerular nephritides, lung fibrosis, restenosis (International Patent Application WO 94/09128), alveolitis (Jones et al., 1992, *J. Immunol.*, 149, 2147) and asthma. Other disease areas where MCP-1 is thought to play a part in their pathology are atherosclerosis (e.g. Koch et al., 1992, *J. Clin. Invest.*, 90, 772 -779), psoriasis (Deleuran et al., 1996, *J. Dermatological Science*, 13, 228-236), delayed-type 15 hypersensitivity reactions of the skin, inflammatory bowel disease (Grimm et al., 1996, *J. Leukocyte Biol.*, 59, 804-812), multiple sclerosis and brain trauma (Berman et al, 1996, *J. Immunol.*, 156, 3017-3023). An MCP-1 inhibitor may also be useful to treat stroke, reperfusion injury, ischemia, myocardial infarction and transplant rejection.

MCP-1 acts through the MCP-1 receptor (also known as the CCR2 receptor). MCP-2 20 and MCP-3 may also act, at least in part, through the MCP-1 receptor. Therefore in this specification, when reference is made to "inhibition or antagonism of MCP-1" or "MCP-1 mediated effects" this includes inhibition or antagonism of MCP-2 and/or MCP-3 mediated effects when MCP-2 and/or MCP-3 are acting through the MCP-1 receptor.

Copending International Patent Application Nos. PCT/GB98/02340 and 25 PCT/GB98/02341 describe and claim groups of compounds based upon the indole ring structure which are inhibitors of MCP-1 and therefore have applications in therapy.

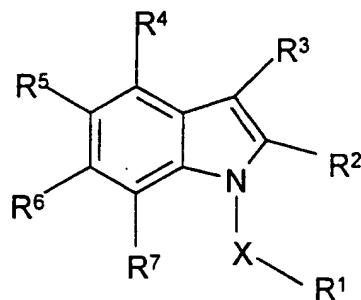
The use of certain indole derivatives as NMDA antagonists is described in USP5051442, WO9312780, EP-483881. Other indoles and their use as inhibitors of leukotriene biosynthesis is described in for example, EP-A- 275-667, EP-A-419049 and USP 30 5,190,968.

More recently, WO 99/33800 describes various indole derivatives as inhibitors of factor XA.

The applicants have found a particular substitution at the 4-position on the indole ring produces advantageous results when used therapeutically as inhibitors of MCP-1.

According to the present invention there is provided a compound of formula (I)

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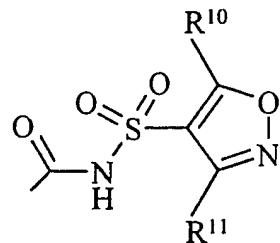
(I)

where:

10 X is CH₂ or SO₂

R¹ is an optionally substituted aryl or heteroaryl ring;

R² is carboxy, cyano, -C(O)CH₂OH, -CONHR⁸, -SO₂NHR⁹, tetrazol-5-yl, SO₃H, or a group of formula (VI)



15

(VI)

where R⁸ is selected from hydrogen, alkyl, aryl, cyano, hydroxy, -SO₂R¹² where R¹² is alkyl, aryl, heteroaryl, or haloalkyl, or R⁸ is a group -(CHR¹³)_r-COOH where r is an integer of 1-3 and each R¹³ group is independently selected from hydrogen or alkyl; R⁹ is hydrogen, alkyl, optionally substituted aryl such as optionally substituted phenyl or optionally substituted

20 heteroaryl such as 5 or 6 membered heteroaryl groups, or a group COR¹⁴ where R¹⁴ is alkyl, aryl, heteroaryl or haloalkyl; R¹⁰ and R¹¹ are independently selected from hydrogen or alkyl, particularly C₁₋₄ alkyl;

R³ is hydrogen, a functional group, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted alkoxy, optionally substituted aralkyl, optionally substituted aralkyloxy, optionally substituted cycloalkyl;

5 R⁴ is a group OR¹⁵ or S(O)_qR¹⁵, where q is 0, 1 or 2 and R¹⁵ is a substituted hydrogen-containing alkyl group; and

R⁵, R⁶ and R⁷ are independently selected from hydrogen, a functional group or an optionally substituted hydrocarbyl group or optionally substituted heterocyclic group.

Suitably, R⁴ is other than a OR¹⁵ where R¹⁵ is C₁₋₄alkyl substituted a single 10 unsubstituted phenyl, such as benzyloxy.

In addition, the invention provides a pharmaceutically acceptable salt, *in vivo* hydrolysable ester, or amide of the compound of formula (I).

Compounds of formula (I) are inhibitors of monocyte chemoattractant protein-1. In addition, they appear to inhibit RANTES induced chemotaxis. RANTES is another 15 chemokine from the same family as MCP-1, with a similar biological profile, but acting though the CCR1 receptor. As a result, these compounds can be used to treat disease mediated by these agents, in particular inflammatory disease. Thus the invention further provides a compound of formula (I) for use in the treatment of inflammatory disease.

In this specification the term 'alkyl' when used either alone or as a suffix includes 20 straight chained, branched structures. These groups may contain up to 10, preferably up to 6 and more preferably up to 4 carbon atoms. Similarly the terms "alkenyl" and "alkynyl" refer to unsaturated straight or branched structures containing for example from 2 to 10, preferably from 2 to 6 carbon atoms. Cyclic moieties such as cycloalkyl, cycloalkenyl and cycloalkynyl are similar in nature but have at least 3 carbon atoms. Terms such as "alkoxy" comprise alkyl 25 groups as is understood in the art.

When it is stated that the an alkyl group is "hydrogen containing", it means that at least one hydrogen atom is present, thus excluding perhaloalkyl groups for example.

The term "halo" includes fluoro, chloro, bromo and iodo. References to aryl groups include aromatic carbocyclic groups such as phenyl and naphthyl. The term "heterocyclyl" 30 includes aromatic or non-aromatic rings, for example containing from 4 to 20, suitably from 5 to 8 ring atoms, at least one of which is a heteroatom such as oxygen, sulphur or nitrogen. Examples of such groups include furyl, thienyl, pyrrolyl, pyrrolidinyl, imidazolyl, triazolyl,

thiazolyl, tetrazolyl, oxazolyl, isoxazolyl, pyrazolyl, pyridyl, pyrimidinyl, pyrazinyl, pyridazinyl, triazinyl, quinolinyl, isoquinolinyl, quinoxalinyl, benzothiazolyl, benzoxazolyl, benzothienyl or benzofuryl.

“Heteroaryl” refers to those groups described above which have an aromatic character.

5 The term “aralkyl” refers to aryl substituted alkyl groups such as benzyl.

Other expressions used in the specification include “hydrocarbyl” which refers to any structure comprising carbon and hydrogen atoms. For example, these may be alkyl, alkenyl, alkynyl, aryl, heterocyclyl, alkoxy, aralkyl, cycloalkyl, cycloalkenyl or cycloalkynyl.

The term “functional group” refers to reactive substituents. They may comprise

10 electron-donating or electron-withdrawing. Examples of such groups include halo, cyano, nitro, $C(O)_nR^{18}$, OR^{18} , $S(O)_mR^{18}$, $NR^{19}R^{20}$, $C(O)NR^{19}R^{20}$, $OC(O)NR^{19}R^{20}$, $-NR^{19}C(O)_nR^{18}$, $-NR^{18}CONR^{19}R^{20}$, $-N=CR^{18}R^{19}$, $S(O)_nNR^{19}R^{20}$ or $-NR^{19}S(O)_mR^{18}$ where R^{18} , R^{19} and R^{20} are independently selected from hydrogen or optionally substituted hydrocarbyl, or R^{19} and R^{20} together form an optionally substituted heterocyclic ring as defined above, which optionally 15 contains further heteroatoms such as sulphur, $S(O)$, SO_2 , oxygen and nitrogen, n is an integer of 1 or 2, m is an integer of 1-3.

Suitable optional substituents for hydrocarbyl groups R^{18} , R^{19} and R^{20} include halo, perhaloalkyl such as trifluoromethyl, mercapto, hydroxy, carboxy, alkoxy, heteroaryl, heteroaryloxy, alkenyloxy, alkynyoxy, alkoxyalkoxy, aryloxy (where the aryl group may be 20 substituted by halo, nitro, or hydroxy), cyano, nitro, amino, mono- or di-alkyl amino, oximino or $S(O)_mR^{16}$ where m' is 1 or 2 and R^{16} is alkyl.

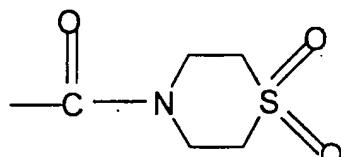
Where R^{19} and R^{20} form a heterocyclic group, this may be optionally substituted by hydrocarbyl such as alkyl as well as those substituents listed above for hydrocarbyl groups.

Suitable substituents for hydrocarbyl or heterocyclic groups R^5 , R^6 and R^7 include 25 those listed above for R^{18} , R^{19} and R^{20} .

Suitably R^1 is an optionally substituted phenyl, pyridyl, naphthyl, furyl or thienyl ring, and in particular is a substituted phenyl or pyridyl ring.

Suitable optional substituents for R^1 in formula (I) include alkyl, alkenyl, alkynyl, halo, haloalkyl including perhaloalkyl such as trifluoromethyl, mercapto, alkoxy, haloalkoxy, 30 alkenyloxy, alkynyoxy, hydroxyalkoxy, alkoxyalkoxy, alkanoyl, alkanoyloxy, cyano, nitro, amino, mono- or di-alkyl amino, oximino, sulphonamido, carbamoyl, mono or dialkylcarbamoyl or $S(O)_mR^{21}$ where m is as defined above and R^{21} is hydrocarbyl.

Particular examples of substituents R⁵, R⁶ and R⁷ include hydrogen, hydroxy, halo, optionally substituted alkyl such as aralkyl, carboxyalkyl or the amide derivative thereof; alkoxy; aryloxy; aralkyloxy; or an amino group which is optionally substituted with alkyl, aryl or aralkyl. A specific functional group which is suitable for R⁵, R⁶ and/or R⁷ is a group 5 of sub-formula (IV).



(IV)

10 Particular examples of groups R⁵, R⁶ and R⁷ are hydrogen, hydroxy, halo or alkoxy. In particular R⁶ and R⁷ are hydrogen. R⁵ may be hydrogen but in addition is suitably a small substituent such as hydroxy, halo or methoxy.

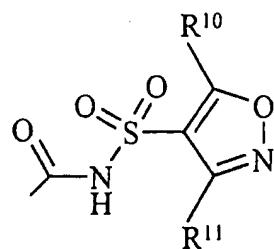
Particular substituents for R¹ include trifluoromethyl, C₁₋₄alkyl, halo, trifluoromethoxy, C₁₋₄alkoxy, C₁₋₄alkanoyl, C₁₋₄alkanoyloxy, nitro, carbamoyl, 15 C₁₋₄alkoxycarbonyl, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphanyl, C₁₋₄alkylsulphonyl, sulphonamido, carbamoylC₁₋₄alkyl, N-(C₁₋₄alkyl)carbamoylC₁₋₄alkyl, N-(C₁₋₄alkyl)₂carbamoyl-C₁₋₄alkyl, hydroxyC₁₋₄alkyl or C₁₋₄alkoxyC₁₋₄alkyl.

Additionally or alternatively, two such substituents together may form a divalent radical of the formula -O(CH₂)₁₋₄O- attached to adjacent carbon atoms on the R¹ ring.

20 Preferred substituents for R¹ are one or more non-polar substituents such as halo.

In particular, R¹ is substituted by one or more halo groups, in particular chlorine. A particular example of an R¹ group is 3,4-dichlorophenyl, 3-fluoro-4-chlorophenyl, 3-chloro-4-fluorophenyl or 2,3-dichloropyrid-5-yl.

Examples of groups R² include carboxy; cyano; tetrazol-5-yl; SO₃H; -CONHR⁸ where 25 R⁸ is selected from cyano, hydroxy, -SO₂R¹² where R¹² is alkyl such as C₁₋₄ alkyl, aryl such as phenyl, heteroaryl or trifluoromethyl, or R⁸ is a group-(CHR¹³)_r-COOH where r is an integer of 1-3 and each R¹³ group is independently selected from hydrogen or alkyl such as C₁₋₄ alkyl; or R² is a group -SO₂NHR⁹ where R⁹ is an optionally substituted phenyl or an optionally substituted 5 or 6 membered heteroaryl group, or a group COR¹⁴ where R¹⁴ is alkyl such as 30 C₁₋₄ alkyl, aryl such as phenyl, heteroaryl or trifluoromethyl, or R² is a group of formula (VI)



(VI)

where R¹⁰ and R¹¹ are independently selected from hydrogen or alkyl, particularly C₁₋₄ alkyl.

5 Preferably R² is carboxy or a pharmaceutically acceptable salt or ester thereof.

Suitable groups R³ include hydrogen, fluoro, chloro, bromo, iodo, methyl, cyano, trifluoromethyl, hydroxymethyl, alkoxyalkyl such as C₁₋₄alkoxymethyl, methoxy, benzyloxy, carboxyalkoxy such as carboxymethoxy, methylsulphanyl, methylsulphinyl, methylsulphonyl or carboxyC₃₋₆cycloalkyl, -(CHR²²)_r-NR²³R²⁴ (where r is 0-2, each R²² is independently 10 hydrogen or alkyl, in particular C₁₋₄ alkyl, R²³ and R²⁴ are independently selected from H and C₁₋₄alkyl or R²³ and R²⁴ together with the nitrogen to which they are attached form a 5 or 6 membered ring optionally containing one further heteroatom selected from O, N, S, S(O) or SO₂. Suitably R²³ and R²⁴ together form a heterocyclic ring such as morpholino or piperazinyl.

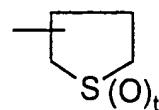
Other such groups R³ include optionally substituted aryl groups, such as optionally 15 substituted phenyl or naphthyl group. Suitable substituents for phenyl groups R³ include one or more groups selected from chlorine, fluorine, methyl, trifluoromethyl, trifluoromethoxy, amino, formyl, phenyl, methoxy, phenoxy or phenyl.

R³ may comprise a range of substituents as listed above, in particular, hydrogen or a small substituent group such as C₁₋₄alkyl in particular methyl, or trifluoromethyl, and is 20 preferably hydrogen.

Suitably R¹⁵ as used in the definition of R⁴ comprises a C₁₋₃alkyl group.

Suitable optional substituents for the group R¹⁵ include one or more groups selected 25 from functional groups as hereinbefore defined, as well as aryl or heterocyclic groups, either of which may themselves be substituted by one or more functional groups. Preferably, when R¹⁵ is an aryl group such as phenyl, the phenyl ring is substituted by for example, a functional group. Preferably, when R¹⁵ has a heteroaryl substituent, it is spaced from the indole ring by more than one CH₂ group, and so the alkyl group R¹⁵ is other than methyl. Most preferably, when R¹⁵ has a heterocyclic substituent, it is non-aromatic, such as morpholino,

tetrahydropyrazinyl where the second nitrogen atom is H, alkyl or hydroxy alkyl substituted, (and in particular is H or alkyl substituted) or a group



where t is 0,1 or 2 and preferably 2.

5 Suitable substitutents for C_{1-3} alkyl groups R^{15} are with one or more groups selected from halo; hydroxy; cyano; amino, mono- or di-alkylamino wherein each alkyl group is optionally substituted by hydroxy, alkoxy or heterocycl; C_{1-4} alkoxy; carboxy; sulphonamido; $CONH_2$; morpholino; tetrahydropyrazinyl which is optionally N-substituted by alkyl or hydroxyalkyl; tetrahydropyridyl optionally substituted by hydroxy or

10 hydroxyalkyl, pyridyl, pyrimidinyl, phenyl optionally substituted by carboxy, halo, hydroxy, alkoxy, carbamoyl, acyl or hydroxyalkyl where the alkyl group suitably includes at least two carbon atoms.

Preferably, when R^{15} is alkyl substituted by phenyl, either the alkyl moiety carries a further substituent as described above, or the phenyl ring is substituted as described above.

15 Particular examples of substituents for groups R^{15} as present in R^4 include one or more groups selected from halo such as chloro, hydroxy, cyano, amino, mono- or di-alkylamino, C_{1-4} alkoxy, carboxy, sulphonamido, $CONH_2$, morpholino, tetrahydropyrazinyl which is optionally N-substituted by alkyl or hydroxy alkyl, pyridyl, pyrimidinyl, phenyl optionally substituted by carboxy, halo such as chloro, hydroxy, alkoxy such as methoxy, carbamoyl, acyl such as acetyl, or hydroxyalkyl where the alkyl group suitably includes at least two carbon atoms, such as hydroxyethyl.

Where R^{15} is a heterocyclic group, these may be substituted by functional groups, or by alkyl groups such as methyl or ethyl, or alkenyl or alkynyl groups any of which may be substituted, for example with hydroxy.

25 A preferred group for R^4 is a group OR^{15} where R^{15} is a straight or branched chain alkyl group which carries at least one hydroxy group, for example 1 or 2 hydroxy groups. Other substituents, as defined above, may be provided on the alkyl chain.

Preferably R^{15} is a group of formula $-(CH_2)_a[(CHOH)(CH_2)_b]_dCH_2OH$ where a is an integer of from 1 to 4, b is 0 or an integer of from 1 to 4, and d is 0 or 1.

30 Examples of such R^{15} include $CH_2CHOHCH_2OH$ and CH_2CH_2OH , $CH_2CH_2CH_2OH$. X is CH_2 or SO_2 and is preferably CH_2 .

Suitable pharmaceutically acceptable salts of compounds of formula (I) include are base salts such as an alkali metal salt for example sodium, an alkaline earth metal salt for example calcium or magnesium, an organic amine salt for example triethylamine, morpholine, *N*-methylpiperidine, *N*-ethylpiperidine, procaine, dibenzylamine, *N,N*-dibenzylethylamine or 5 amino acids for example lysine. In another aspect, where the compound is sufficiently basic, suitable salts include acid addition salts such as methanesulfonate, fumarate, hydrochloride, hydrobromide, citrate, maleate and salts formed with phosphoric and sulphuric acid. There may be more than one cation or anion depending on the number of charged functions and the valency of the cations or anions. A preferred pharmaceutically acceptable salt is a sodium salt.

10 An *in vivo* hydrolysable ester of a compound of the formula (I) containing carboxy or hydroxy group is, for example, a pharmaceutically acceptable ester which is hydrolysed in the human or animal body to produce the parent acid or alcohol.

Suitable pharmaceutically acceptable esters for carboxy include C_{1-6} alkyl esters such as methyl or ethyl esters, C_{1-6} alkoxymethyl esters for example methoxymethyl, 15 C_{1-6} alkanoyloxymethyl esters for example pivaloyloxymethyl, phthalidyl esters, C_{3-8} cycloalkoxy-carbonyloxy C_{1-6} alkyl esters for example 1-cyclohexylcarbonyloxyethyl; 1,3-dioxolen-2-onylmethyl esters for example 5-methyl-1,3-dioxolen-2-onylmethyl; and C_{1-6} alkoxycarbonyloxyethyl esters for example 1-methoxycarbonyloxyethyl and may be formed at any carboxy group in the compounds of this invention.

20 An *in vivo* hydrolysable ester of a compound of the formula (I) containing a hydroxy group includes inorganic esters such as phosphate esters and α -acyloxyalkyl ethers and related compounds which as a result of the *in vivo* hydrolysis of the ester breakdown to give the parent hydroxy group. Examples of α -acyloxyalkyl ethers include acetoxymethoxy and 2,2-dimethylpropionyloxymethoxy. A selection of *in vivo* hydrolysable ester forming groups 25 for hydroxy include alkanoyl, benzoyl, phenylacetyl and substituted benzoyl and phenylacetyl, alkoxy carbonyl (to give alkyl carbonate esters), dialkylcarbamoyl and *N*-(dialkylaminoethyl)-*N*-alkylcarbamoyl (to give carbamates), dialkylaminoacetyl and carboxyacetyl.

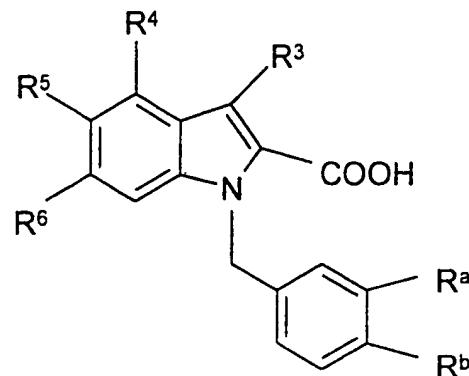
Suitable amides include, for example, a N - C_{1-6} alkyl and N,N -di-30 (C_{1-6} alkyl)amide such as N -methyl, N -ethyl, N -propyl, N,N -dimethyl, N -ethyl- N -methyl or N,N -diethylamide.

Esters which are not *in vivo* hydrolysable are useful as intermediates in the production of the compounds of formula (I) and therefore these form a further aspect of the invention.

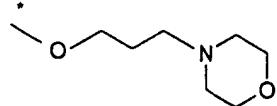
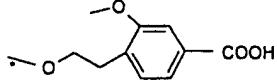
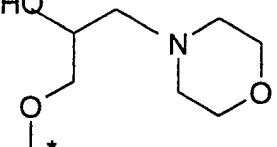
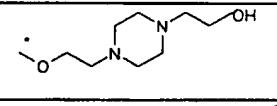
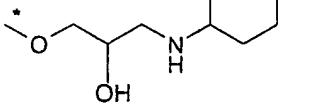
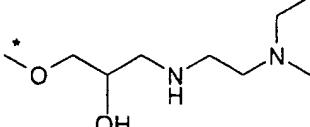
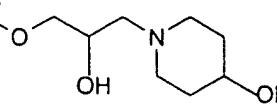
Thus examples of compounds of formula (I) include the following:

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Table 1



Compd No.	R ³	R ⁴	R ⁵	R ⁶	R ^a	R ^b
1	H	OCH ₂ CH ₂ OH	H	H	H	H
2	H	OCH ₂ CH ₂ OCH ₃	H	H	H	H
3	H		H	H	H	H
4	H		H	H	H	H
5	H	OCH ₂ COOH	H	H	F	F
6	H	OCH ₂ COOH	H	H	Cl	H
7	H		H	H	H	H
8	H	OCH ₂ COOH	H	H	H	Cl
9	H	OCH ₂ COOH	OCH ₃	H	Cl	Cl

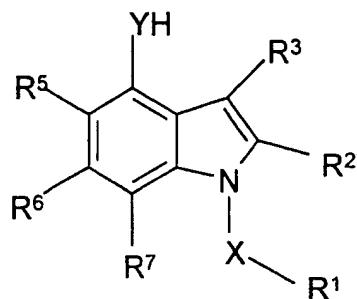
10	H		OCH ₃	H	Cl	Cl
11	H	OCH ₂ CONH ₂	OCH ₃	H	Cl	Cl
12	H	OCH(CH ₃)COOH	H	H	Cl	Cl
13	H		H	H	Cl	Cl
14	H	O(CH ₂) ₂ OH	H	H	Cl	Cl
15	H	O(CH ₂) ₃ OH	H	H	Cl	Cl
16	H	OCH ₂ CH(OH)CH ₂ N(CH ₃) ₂	H	H	Cl	Cl
17	H		H	H	Cl	Cl
18	H	O(CH ₂) ₂ N(CH ₃) ₂	H	H	Cl	Cl
19	H	S(CH ₂) ₃ OH	H	H	Cl	Cl
20	H	S(O) ₂ (CH ₂) ₃ OH	H	H	Cl	Cl
21	H	OCH ₂ CHOHCH ₂ OH	H	H	Cl	Cl
22	H		H	H	Cl	Cl
23	H		H	H	Cl	Cl
24	H		H	H	Cl	Cl
25	H		H	H	Cl	Cl

26	H		H	H	Cl	Cl
27	H		H	H	Cl	Cl
28	H		H	H	Cl	Cl

where * indicates the point of attachment of the group to the indole ring.

Compounds of formula (I) are suitably prepared by methods such methods analogous to those described in International Patent Application Nos. PCT/GB98/02340 and 5 PCT/GB98/02341.

In particular compounds of formula (I) can be prepared by reacting a compound of formula (VII)



10

(VII)

where R¹, R³ R⁵, R⁶, R⁷ and X are as defined in relation to formula (I), R^{2'} is a group R² as defined in relation to formula (I) or a protected form thereof and Y is oxygen or sulphur with a compound of formula (VIII)

15

Z-R^{15'}

(VIII)

where Z is a leaving group and $R^{15'}$ is a group R^{15} as defined in claim 1 or a precursor thereof, and thereafter if desired or necessary carrying out one or more of the following steps:

- (i) converting a precursor group $R^{15'}$ to a group R^{15} ;
- (ii) converting a group R^{15} to another such group;
- 5 (iii) oxidising a thiol group R^4 to a sulphinyl or sulphonyl group;
- (iv) deprotecting a group $R^{2'}$ or converting the existing group R^2 to a different R^2 group.

The reaction between the compounds of formula (VII) and (VIII) is suitably effected in an organic solvent such as dimethylformamide (DMF) in the presence of a base such as a 10 hydride or carbonate salt, in particular sodium hydride or potassium carbonate. Temperatures in the range of from 0° to 100°C are suitably employed. Examples of suitable leaving groups Z include halogen such as chloro and bromo.

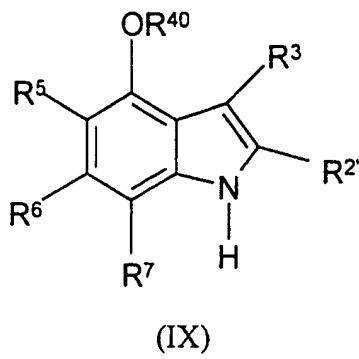
In general, the compound of formula (VII) will comprise a compound where R^2 is an ester group. Deesterification in optional step (iv) using conventional methods as illustrated 15 hereinafter will yield the corresponding compound of formula (I) where R^2 is a carboxylic acid group.

Optional step (i) above may be carried out using conventional methods, depending upon the precise nature of the precursor involved. For example, a precursor to a di-substituted alkyl group R^{15} may be an epoxide. Addition of an amine to the epoxide, for 20 example as illustrated hereinafter will result in the production of a compound of formula (I) where R^{15} carries both a hydroxy and an amine substituent. Many other possible reactions of this nature would be apparent to a chemist.

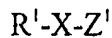
Similarly, optional step (ii) may be effected using conventional methods. For example, halo substituents may be replaced by others using a nucleophilic displacement 25 reaction. Again, examples of such reactions are given hereinafter, but many others would be readily apparent.

Oxidation in optional step (iii) is suitably effected using an appropriate oxidising agent. For instance hydrogen peroxide may be reacted with a thiol to produce the corresponding sulphonyl compound of formula (I).

30 Compounds of formula (VII) can be prepared in various ways depending upon the nature of the group Y. For instance, when Y is oxygen, the compound of formula (VII) may be prepared by reacting a compound of formula (IX)



where R^3 , R^5 , R^6 and R^7 are as defined in relation to formula (I), $R^{2'}$ is as defined in relation to formula (VII) and R^{40} is a protecting group such as acetyl or benzyl; with compound of formula (X)



10



where R^1 and X are as defined in relation to formula (I) and Z^1 is a leaving group, and thereafter removing the protecting group R^{40} .

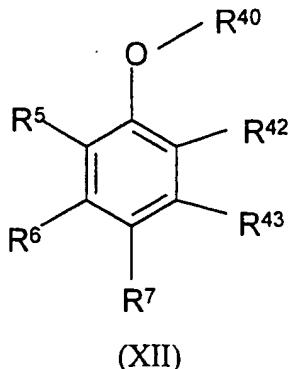
Suitable leaving groups for Z include halide such as chloride, bromide or iodide, as well as mesylate or tosylate. The reaction is suitably effected in an organic solvent such as 15 dimethylformamide (DMF) tetrahydrofuran (THF) or DCM in the presence of a base such as sodium hydride, sodium hydroxide, potassium carbonate. Optionally the reaction is effected in the presence of a suitable phase transfer catalyst. The choice of base and solvent is interdependent to a certain extent in that certain solvents are compatible with some bases only as is understood in the art. For example, sodium hydride may preferably be used with 20 dimethylformamide or tetrahydrofuran and sodium hydroxide is preferably used with dichloromethane and a phase transfer catalyst.

The reaction can be carried out at moderate temperatures, for example from 0 to 50°C and conveniently at about ambient temperature.

Preferably, $R^{2'}$ is an ester group in the compound of formula IX and this may be 25 subsequently converted to an acid or to another ester or salt, by conventional methods later in the process. For example, when X is a group SO_2 and R^2 is a methyl ester of carboxy, it may be converted to the corresponding carboxylic acid by reaction with lithium iodide in dry pyridine or DMF.

The reaction conditions employed in the deprotection step to remove R⁴⁰ will depend upon the nature of the protecting group R⁴⁰ and would be apparent to a skilled person. Acetyl groups may be removed by reaction with a strong base such as sodium methoxide, whereas benzyl groups may be removed by hydrogenation, for example in the presence of a palladium 5 catalyst.

Compounds of formula (IX) may be prepared by cyclisation of a compound of formula (XII)



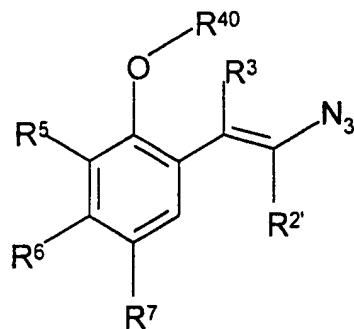
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where R⁵, R⁶, R⁷ and R⁴⁰ are as defined above and R⁴² and R⁴³ represent a combination of moieties which can cyclise to form an appropriately substituted pyrrole ring. For example, R⁴² can be a group of formula -CH=C(R⁴⁴)N, where R⁴⁴ is a group R² as defined above, or a protected form thereof, and R⁴³ may be hydrogen. Cyclisation to form a compound of 15 formula (XII) may then be effected by heating for example under reflux in an organic solvent, in particular a high boiling aprotic solvent such as xylene or toluene.

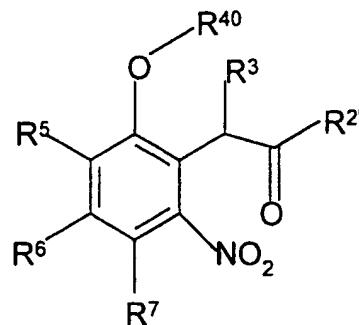
Alternatively, R⁴³ may be nitro and R⁴² may be a group of formula -CH₂C(O)R^{2'} where R^{2'} is as defined above in relation to formula (VII). These compounds will cyclise in the presence of a catalyst such as palladium on carbon in the presence of hydrogen. The reaction 20 may be effected at moderate temperatures for example of from 0 to 80°C, conveniently at about ambient temperature.

Thus examples of compounds of formula (XII) include compounds of formula (XIII) and (XIV)

-15-



(XIII)

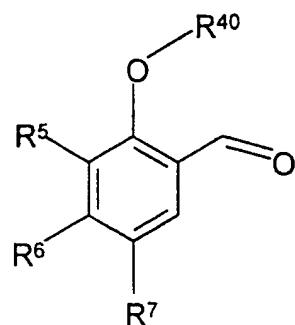


(XIV)

where R^2 , R^3 , R^5 , R^6 , R^7 and R^{40} are as defined above.

Compounds of formula (XIII) where R^3 is hydrogen may be prepared for example by reacting a compound of formula (XV)

10



(XV)

with a compound of formula (XVI)

15



(XVI)

where R^5 , R^6 , R^7 and R^2' are as defined hereinbefore. The reaction may be effected in an organic solvent such as ethanol at low temperatures of from -20 to 0°C, suitably at about 0°C. The reaction is suitably effected in the presence of a base such as an alkoxide, in particular an ethoxide, for example potassium ethoxide.

5 Where necessary or desired, a group R^3 which is other than hydrogen may be added using conventional methods, later in the process.

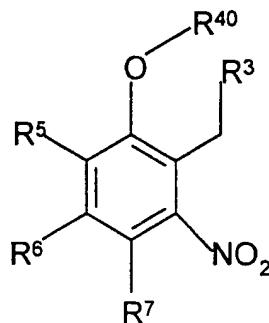
Compounds of formula (XVI) are suitably prepared by reacting a compound of formula (XVII)



10 (XVII)

where R^2' is as defined above and R^{47} is a leaving group such as halide and in particular bromide, with an azide salt, such as an alkali metal azide salt in particular sodium azide.

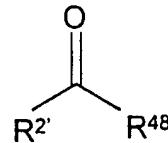
Compounds of formula (XIV) may be prepared by reacting a compound of formula (XVIII)



15

(XVIII)

where R^5 , R^6 , R^7 , R^3 and R^{40} are as defined above, with a compound of formula (XIX)



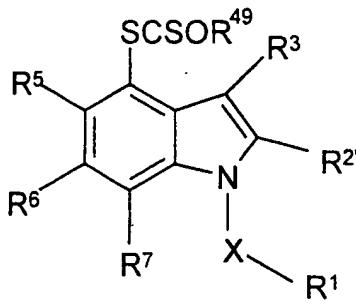
20

(XIX)

where R^2' is as defined above and R^{48} is a leaving group such as alkoxy. Examples of compounds of formula (XIX) are oxalates such as diethyloxalate. The reaction is suitably effected in the presence of a base such as sodium hydride in an organic solvent such as THF. Moderate temperatures of from 0° to 40°C and conveniently ambient temperature is employed.

25

Compounds of formula (VII) where Y is sulphur may conveniently be prepared using alternative methods. For example, they may be prepared by reacting a compound of formula (XX)

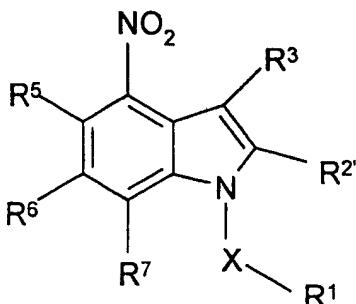


5

(XX)

where R¹, R³ R⁵, R⁶, R⁷ and R^{2'} are as defined above and R⁴⁹ is an alkyl group such as ethyl, with an amine such as ethylene diamine. The reaction is suitably effected in a solvent such as tetrahydrofuran at moderate temperatures from example from 0° to 50°C, conveniently at 10 ambient temperature.

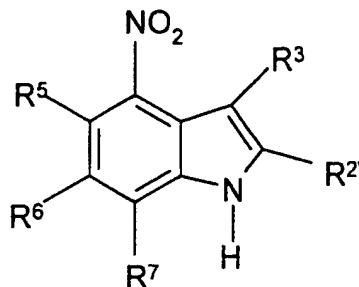
Compounds of formula (XX) are suitably derived from a compound of formula (XXI)



(XXI)

where R¹, R³ R⁵, R⁶, R⁷ and R^{2'} are as defined above, by a sequence of reactions involving for 15 example, the conversion of the nitro group to an amino group, then to a diazonium group and subsequently to a xanthyl group. Suitable reaction conditions for these steps would be apparent from the literature and are illustrated hereinafter.

Compounds of formula (XXI) are suitably formed by reacting a compound of formula (XXII)



(XXII)

with a compound of formula (X) as defined above using conditions similar to those described for the reaction of compound (IX) with compound (X).

5 Compounds of formula (X), (XV), (XVI), (XVII), (XVIII), (XIX) and (XXII) are either known compounds or they can be prepared from known compounds by conventional methods.

According to a further aspect of the invention there is provided a compound of the formula (I) as defined herein, or a pharmaceutically acceptable salt or an *in vivo* hydrolysable 10 ester thereof, for use in a method of treatment of the human or animal body by therapy. In particular, the compounds are used in methods of treatment of inflammatory disease.

According to a further aspect of the present invention there is provided a method for antagonising an MCP-1 mediated effect in a warm blooded animal, such as man, in need of such treatment, which comprises administering to said animal an effective amount of a 15 compound of formula (I), or a pharmaceutically acceptable salt, or an *in vivo* hydrolysable ester thereof.

The invention also provides a pharmaceutical composition comprising a compound of formula (I) as defined herein, or a pharmaceutically acceptable salt, or an *in vivo* hydrolysable ester thereof, in combination with a pharmaceutically acceptable carrier.

20 The compositions of the invention may be in a form suitable for oral use (for example as tablets, lozenges, hard or soft capsules, aqueous or oily suspensions, emulsions, dispersible powders or granules, syrups or elixirs), for topical use (for example as creams, ointments, gels, or aqueous or oily solutions or suspensions), for administration by inhalation (for example as a finely divided powder or a liquid aerosol), for administration by insufflation (for 25 example as a finely divided powder) or for parenteral administration (for example as a sterile aqueous or oily solution for intravenous, subcutaneous, intramuscular or intramuscular dosing or as a suppository for rectal dosing).

The compositions of the invention may be obtained by conventional procedures using conventional pharmaceutical excipients, well known in the art. Thus, compositions intended for oral use may contain, for example, one or more colouring, sweetening, flavouring and/or preservative agents.

5 Suitable pharmaceutically acceptable excipients for a tablet formulation include, for example, inert diluents such as lactose, sodium carbonate, calcium phosphate or calcium carbonate, granulating and disintegrating agents such as corn starch or algenic acid; binding agents such as starch; lubricating agents such as magnesium stearate, stearic acid or talc; preservative agents such as ethyl or propyl *p*-hydroxybenzoate, and anti-oxidants, such as 10 ascorbic acid. Tablet formulations may be uncoated or coated either to modify their disintegration and the subsequent absorption of the active ingredient within the gastrointestinal track, or to improve their stability and/or appearance, in either case, using conventional coating agents and procedures well known in the art.

Compositions for oral use may be in the form of hard gelatin capsules in which the 15 active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules in which the active ingredient is mixed with water or an oil such as peanut oil, liquid paraffin, or olive oil.

Aqueous suspensions generally contain the active ingredient in finely powdered form together with one or more suspending agents, such as sodium carboxymethylcellulose, 20 methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents such as lecithin or condensation products of an alkylene oxide with fatty acids (for example polyoxyethylene stearate), or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters 25 derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and 30 hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives (such as ethyl or propyl *p*-hydroxybenzoate,

anti-oxidants (such as ascorbic acid), colouring agents, flavouring agents, and/or sweetening agents (such as sucrose, saccharine or aspartame).

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil (such as arachis oil, olive oil, sesame oil or coconut oil) or in a mineral oil (such as liquid paraffin). The oily suspensions may also contain a thickening agent such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set out above, and flavouring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by 10 the addition of water generally contain the active ingredient together with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients such as sweetening, flavouring and colouring agents, may also be present.

The pharmaceutical compositions of the invention may also be in the form of 15 oil-in-water emulsions. The oily phase may be a vegetable oil, such as olive oil or arachis oil, or a mineral oil, such as for example liquid paraffin or a mixture of any of these. Suitable emulsifying agents may be, for example, naturally-occurring gums such as gum acacia or gum tragacanth, naturally-occurring phosphatides such as soya bean, lecithin, an esters or partial esters derived from fatty acids and hexitol anhydrides (for example sorbitan monooleate) and 20 condensation products of the said partial esters with ethylene oxide such as polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening, flavouring and preservative agents.

Syrups and elixirs may be formulated with sweetening agents such as glycerol, propylene glycol, sorbitol, aspartame or sucrose, and may also contain a demulcent, 25 preservative, flavouring and/or colouring agent.

The pharmaceutical compositions may also be in the form of a sterile injectable aqueous or oily suspension, which may be formulated according to known procedures using one or more of the appropriate dispersing or wetting agents and suspending agents, which have been mentioned above. A sterile injectable preparation may also be a sterile injectable 30 solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example a solution in 1,3-butanediol.

Suppository formulations may be prepared by mixing the active ingredient with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Suitable excipients include, for example, cocoa butter and polyethylene glycols.

5 Topical formulations, such as creams, ointments, gels and aqueous or oily solutions or suspensions, may generally be obtained by formulating an active ingredient with a conventional, topically acceptable, vehicle or diluent using conventional procedure well known in the art.

10 Compositions for administration by insufflation may be in the form of a finely divided powder containing particles of average diameter of, for example, 30μ or much less, the powder itself comprising either active ingredient alone or diluted with one or more physiologically acceptable carriers such as lactose. The powder for insufflation is then conveniently retained in a capsule containing, for example, 1 to 50mg of active ingredient for use with a turbo-inhaler device, such as is used for insufflation of the known agent sodium 15 cromoglycate.

20 Compositions for administration by inhalation may be in the form of a conventional pressurised aerosol arranged to dispense the active ingredient either as an aerosol containing finely divided solid or liquid droplets. Conventional aerosol propellants such as volatile fluorinated hydrocarbons or hydrocarbons may be used and the aerosol device is conveniently arranged to dispense a metered quantity of active ingredient.

For further information on Formulation the reader is referred to Chapter 25.2 in Volume 5 of Comprehensive Medicinal Chemistry (Corwin Hansch; Chairman of Editorial Board), Pergamon Press 1990.

25 The amount of active ingredient that is combined with one or more excipients to produce a single dosage form will necessarily vary depending upon the host treated and the particular route of administration. For example, a formulation intended for oral administration to humans will generally contain, for example, from 0.5 mg to 2 g of active agent compounded with an appropriate and convenient amount of excipients which may vary from about 5 to about 98 percent by weight of the total composition. Dosage unit forms will 30 generally contain about 1 mg to about 500 mg of an active ingredient. For further information on Routes of Administration and Dosage Regimes the reader is referred to Chapter 25.3 in

Volume 5 of Comprehensive Medicinal Chemistry (Corwin Hansch; Chairman of Editorial Board), Pergamon Press 1990.

The size of the dose for therapeutic or prophylactic purposes of a compound of the Formula I will naturally vary according to the nature and severity of the conditions, the age 5 and sex of the animal or patient and the route of administration, according to well known principles of medicine. As mentioned above, compounds of the Formula I are useful in treating diseases or medical conditions which are due alone or in part to the effects of farnesylation of rats.

In using a compound of the Formula I for therapeutic or prophylactic purposes it will 10 generally be administered so that a daily dose in the range, for example, 0.5 mg to 75 mg per kg body weight is received, given if required in divided doses. In general lower doses will be administered when a parenteral route is employed. Thus, for example, for intravenous administration, a dose in the range, for example, 0.5 mg to 30 mg per kg body weight will generally be used. Similarly, for administration by inhalation, a dose in the range, for 15 example, 0.5 mg to 25 mg per kg body weight will be used. Oral administration is however preferred.

A further aspect of the invention comprises a compound of formula (I) as defined above, or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof, for use in the preparation of a medicament for the treatment of inflammatory disease.

20 The invention is further illustrated, but not limited by the following Examples in which the following general procedures were used unless stated otherwise.

Preparation 1

Ethyl N-(3,4-dichlorobenzyl)-4-nitroindole-2-carboxylate

25 Ethyl 4-nitroindole-2-carboxylate (26 g) [prepared according to S. M. Parmerter *et. al.* *J. Amer. Chem. Soc.*, 1958, **80**, 4621], 3,4-dichlorobenzyl chloride (16 ml), potassium carbonate (17 g) and potassium iodide (2 g) in DMF (250 ml) were stirred at 60°C for 2 hours. The reaction was concentrated *in vacuo* and the residue partitioned between water and dichloromethane. Iso-hexane was added to the combined organic extracts resulting in 30 crystallisation of the product as yellow needles (39 g, 89%) NMR d (CD₃SOCD₃) 1.30 (t, 3H), 4.32 (q, 2H), 5.93 (s, 2H), 6.88 (dd, 1H), 7.18 (d, 1H), 7.52 (d, 1H), 7.56 (dd, 1H), 7.78 (s, 1H), 8.17 (m, 2H); M/z (+)395 (MH⁺), 393.

Preparation 2**Ethyl N-(3,4-dichlorobenzyl)-4-aminoindole-2-carboxylate**

A solution of ethyl *N*-(3,4-dichlorobenzyl)-4-nitroindole-2-carboxylate (2.41 g) in 5 tetrahydrofuran (100 ml) was stirred in the presence of titanium trichloride (15% aqueous solution, 50 ml) at room temperature overnight. The reaction was treated with 40% sodium hydroxide solution and extracted with 5% methanol in dichloromethane. Combined organic extracts were dried (MgSO_4) and concentrated *in vacuo* to give the product as a brown solid (1.98 g, 89%); NMR d (CD_3SOCD_3) 1.3 (t, 3H), 4.2 (q, 2H), 5.7 (s, 4H), 6.2 (d, 1H), 6.6 (d, 10 1H), 7.0 (m, 2H), 7.25 (m, 1H), 7.5 (d, 1H), 7.6 (m, 1H); M/z (+) 363.3 ($M\text{H}^+$).

Preparation 3***N*-(3,4-dichlorobenzyl)-2-ethoxycarbonylindole-4-diazonium tetrafluoroborate**

Ethyl *N*-(3,4-dichlorobenzyl)-4-aminoindole-2-carboxylate (1.98 g) was stirred in 15 water (38 ml) and HCl (2M, 15 ml) at 0°C and a solution of sodium nitrite (940 mg) in water (20 ml) was added dropwise over 10 minutes. This was followed by the addition of concentrated HCl (15 ml). The reaction was stirred for 1 hour at 0°C and a saturated solution of sodium tetrafluoroborate (12 ml) was added. The resulting precipitate was collected by filtration, washed with water and dried *in vacuo* over phosphorous pentoxide to give the 20 product as a light brown solid (2.51g, 100%); NMR d (CD_3SOCD_3) 1.3 (t, 3H), 4.4 (q, 2H), 6.0 (s, 2H), 6.95 (m, 1H), 7.3 (m, 1H), 7.5 (d, 1H), 7.8 (t, 1H), 8.0 (m, 1H), 8.7 (m, 2H); M/z (+) 348.6 [$M\text{-N}_2\text{BF}_4^+$].

Preparation 4**Ethyl S-[*N*-(3,4-dichlorobenzyl)-2-ethoxycarbonylindol-4-yl] dithiocarbamate**

N-(3,4-dichlorobenzyl)-2-ethoxycarbonylindole-4-diazonium tetrafluoroborate (2.5g) was added portionwise over 10 minutes to a stirred solution of potassium ethyl xanthate (0.96 g) in acetone (60 ml) under argon at 0°C. The reaction mixture was allowed to warm to room temperature, poured into 50% brine (200 ml) and extracted with ethyl acetate. Combined 30 organic extracts were dried (MgSO_4), concentrated *in vacuo* and the residue purified by column chromatography using *iso*-hexane : 5% ethyl acetate to 15% ethyl acetate as eluent to give the product as a pale yellow oil (1 g, 39%); NMR d (CD_3SOCD_3) 1.1 (t, 3H), 1.3 (t, 3H),

4.3 (q, 2H), 4.55 (q, 2H), 5.9 (s, 2H), 6.9 (m, 1H), 7.4 (m, 4H), 7.5 (m, 1H), 7.8 (d, 1H); *M/z* (+) 468.3 (MH^+).

Preparation 5

5 Ethyl N-(3,4-dichlorobenzyl)-4-mercaptopindole-2-carboxylate

Ethylene diamine (0.12 ml) was added to a solution of ethyl S-[N-(3,4-dichlorobenzyl-2-ethoxycarbonylindol-4-yl] dithiocarbamate (730 mg) in tetrahydrofuran (25 ml) and stirring was continued for 16 hours. The reaction mixture was concentrated *in vacuo* and the residue partitioned between water and dichloromethane. Combined organic extracts were dried

10 ($MgSO_4$) and concentrated *in vacuo* and the residue purified by column chromatography using *iso*-hexane : 10% ethyl acetate to 20% ethyl acetate as eluent to give the product as a pale yellow oil (400 mg, 67%); NMR d (CD_3SOCD_3) 1.25 (t, 3H), 4.3 (q, 2H), 5.8 (s, 2H), 6.85 (m, 1H), 7.25 (s, 1H), 7.3 (m, 2H), 7.4 (m, 1H), 7.5 (d, 1H), 7.6 (d, 1H); *M/z* (+) 379 (MH^+).

15 Preparation 6

2-Benzyl-3-methoxybenzaldehyde

Benzyl bromide (8.6 ml) was added to a stirred solution of 3-methoxysalicylaldehyde (10 g), potassium carbonate (14.76 g) and potassium iodide (0.12 g) in DMF (120 ml) under an atmosphere of argon. The reaction was heated at 70°C for 16 hours. Water (200 ml) was 20 added causing the precipitation of a brown oil which crystallised on cooling. The solid was filtered, washed with water, dissolved in dichloromethane and dried ($MgSO_4$). The solvent was removed *in vacuo* to give an oil which crystallised on trituration with *iso*-hexane to give the product as an off white solid (12.85 g, 81%); NMR d (CD_3SOCD_3) 3.91 (s, 3H), 5.15 (s, 2H), 7.14 - 7.22 (m, 2H), 7.29 - 7.42 (m, 6H), 10.10 (t, 1H); *M/z* (+) 243 (MH^+).

25

Preparation 7

Methyl azidoacetate

Methyl bromoacetate (62 ml) was added to a suspension of sodium azide (44 g) in DMF (500 ml) at ambient temperature and the reaction stirred for 16 hours. The reaction was 30 poured into ice-water (1000 g) and extracted with ether. Combined organic extracts were washed with water and brine, dried ($MgSO_4$) and concentrated to give the product as a

colourless oil which was used without further purification (64.2 g, 82%); NMR d (CDCl₃) 3.80 (3H, s), 3.90 (2H, s).

Preparation 7a

5 Methyl azidoacetate (toluene solution)

To a stirred solution of methyl bromoacetate (50 ml) and tetra-*n*-butylammonium hydrogen sulphate (3.4 g) in toluene (200 ml) at 20-25°C was added dropwise a solution of sodium azide (36 g) and sodium carbonate (2.0 g) in water (150 ml) over 30 minutes. The mixture was stirred for a further hour, the organic layer separated and dried (MgSO₄) to give 10 the product as a *ca.* 2M toluene solution (250 ml). The product was used without further characterisation.

Preparation 8

Methyl 4-benzyloxy-5-methoxyindole-2-carboxylate

15 A solution of 2-benzyl-3-methoxybenzaldehyde (6 g) and methyl azido acetate (4.27 g) in methanol (45 ml) was added dropwise to a stirred solution of sodium methoxide (2.0 g) in methanol (60 ml) under argon at -40°C. The reaction was then stirred and allowed to warm to room temperature over 16 hours. The reaction was poured into ice water (100 ml) and extracted with diethyl ether. Combined organic extracts were washed with water, dried 20 (MgSO₄) and carefully concentrated *in vacuo*. The residue was dissolved in xylene (15 ml) and added dropwise to a stirred flask of xylene (25 ml) at 160°C. The reaction was stirred for 1 hour then concentrated *in vacuo* and the residue purified by column chromatography using 35% diethyl ether : *iso*-hexane as eluent to give the product as a yellow solid (1.39 g, 42%); NMR d (CD₃SOCD₃) 3.79 (s, 3H), 3.82 (s, 3H), 5.18 (s, 2H), 7.10 (m, 2H), 7.30 - 7.40 (m, 25 6H), 11.78 (brs, 1H); *M/z* (+) 312 (MH⁺).

Preparation 9

Methyl 2-(2-benzyloxyphenyl)-1-azidoacrylate

To a commercial solution of 25% w/w sodium methoxide in methanol (500 ml) was 30 added methanol (1 litre) and the mixture cooled to -20°C with stirring. A solution of 2-benzyloxybenzaldehyde (125 g) and methyl azidoacetate (253 g) in toluene (1 litre) was added dropwise such that the reaction temperature did not exceed 0°C. The mixture was stirred for a

further 3 hours at 0°C and the pale yellow crystals filtered off and washed successively with cold methanol (500 ml) and 1N aqueous acetic acid (500 ml) to give the product (150 g, 82%); NMR d (CDCl₃) 3.88 (s, 3H), 5.15 (s, 2H), 6.92 (d, 1H), 7.00 (dd, 1H), 7.3 - 7.4 (m, 6H), 7.50 (s, 1H), 8.20 (dd, 1H).

5

Preparation 10

Methyl 4-benzyloxyindole-2-carboxylate

A solution of methyl 2-(2-benzyloxyphenyl)-1-azidoacrylate (50 g) in *o*-xylene (500 ml) was added dropwise to refluxing *o*-xylene (200 ml) with stirring, nitrogen was evolved 10 during 30 minutes. The pale yellow solution was allowed to cool and the resulting crystals were filtered and washed with toluene (200 ml) and then hexane (200 ml) to give the product as white needles (39 g, 86%). NMR d (CD₃SOCD₃) 3.83 (s, 3H), 5.22 (s, 2H), 6.61 (d, 1H), 7.03 (d, 1H), 7.1 - 7.2 (m, 2H), 7.3 - 7.5 (m, 5H), 11.9 (brs, 1H).

15

Preparation 11

Methyl 4-hydroxy-5-methoxyindole-2-carboxylate

5% Palladium on carbon (0.3 g) and methyl 4-benzyloxy-5-methoxyindole-2-carboxylate (2.0 g) in ethyl acetate (30 ml) were vigorously stirred under an atmosphere of hydrogen for 16 hours. The reaction was filtered through celite and the concentrated *in vacuo* 20 to give the product as a cream solid (1.29 g, 91%); NMR d (CD₃SOCD₃) 3.74 (s, 3H), 3.82 (s, 3H), 6.80 (d, 1H), 7.00 (d, 1H), 7.16 (s, 1H), 9.10 (s, 1H), 11.58 (brs, 1H); *M/z* (+) 222 (MH⁺).

Preparation 12

Methyl 4-acetoxyindole-2-carboxylate

25 A solution of methyl 4-benzyloxyindole-2-carboxylate (48 g) in ethyl acetate (1 litre) at 50-60°C was hydrogenated over 5%Pd-C catalyst (4.0 g) at 1 atmosphere pressure for 6 hours, until 3.7 litres of hydrogen was absorbed. The catalyst was filtered off and washed with warm ethyl acetate(100 ml). To the combined filtrates was added acetic anhydride (40 ml) and 4-dimethylaminopyridine (1.0 g), and the solution was stirred for 1 hour at 25°C. 30 Ethanol (15 ml) was added and the solution reduced to half volume by evaporation *in vacuo*, then hexane (1 litre) was added to cause crystallisation of the product as white needles (37 g,

93%). NMR d (CD₃SOCD₃) 2.34 (s, 3H), 3.85 (s, 3H), 6.80 (d, 1H), 7.06 (s, 1H), 7.23 (t, 1H), 7.29 - 7.35 (m, 1H), 12.1 (bs, 1H); *M/z* (-) 232 (M-H⁺).

Preparation 13

5 Methyl 4-hydroxyindole-2-carboxylate

Boron tribromide (73.1 ml, 1.0 M solution in DCM) was added dropwise to a solution of methyl 4-methoxyindole-2-carboxylate (5 g) in DCM (200 ml) cooled to -78°C under argon. The reaction was allowed to warm to room temperature then partitioned between dichloromethane and saturated aqueous sodium hydrogen carbonate solution. Combined 10 organic extracts were dried (MgSO₄) and concentrated *in vacuo* and the residue purified by column chromatography using isohexane-50% ethyl acetate as eluent to give the end product as a yellow solid (2.98 g, 64%); NMR d (CD₃SOCD₃) 3.82 (s, 3H), 6.36 (d, 1H), 6.85 (d, 1H), 7.02 (t, 1H), 7.17 (d, 1H), 9.66 (s, 1H), 11.72 (bs, 1H); *M/z* (+) 192 (MH⁺).

15 Preparation 14

Methyl 4-acetoxy-5-methoxyindole-2-carboxylate

A stirred solution of methyl 4-hydroxy-5-methoxyindole-2-carboxylate (0.51 g) and DMAP (10 mg) in acetic anhydride (5 ml) was heated at 80°C for 4 hours. The reaction was concentrated *in vacuo* and the residue dissolved in dichloromethane and washed with 20 hydrochloric acid (2.0 M), saturated aqueous sodium hydrogen carbonate solution, water, aqueous saturated sodium chloride solution and dried (MgSO₄). The solution was concentrated *in vacuo* and the residue was purified by column chromatography using 50% diethyl ether : *iso*-hexane as eluent to give the product as a white solid (0.58 g, 75%); NMR d (CD₃SOCD₃) 2.34 (s, 3H), 3.75 (s, 3H), 3.85 (s, 3H), 6.99 (d, 1H), 7.19 (d, 1H), 7.29 (d, 1H), 25 11.93 (brs, 1H); *M/z* (+) 264 (MH⁺).

Preparation 15

The procedure described in the Preparation 14 above was repeated using the appropriate hydroxyindole. Thus was obtained the compound described below.

Methyl 4-acetoxyindole-2-carboxylate

72% yield; NMR d (CD₃SOCD₃) 2.34 (s, 3H), 3.85 (s, 3H), 6.80 (d, 1H), 7.06 (s, 1H), 7.23 (t, 1H), 7.29 - 7.35 (m, 1H), 12.1 (bs, 1H); *M/z* (-) 232 (M-H⁺).

5 Preparation 16**Methyl 4-acetoxy-N-(3,4-dichlorobenzyl)-5-methoxyindole-2-carboxylate**

3,4-Dichlorobenzyl bromide (1.02 g) was added to a stirred solution of methyl 4-acetoxy-5-methoxyindole-2-carboxylate (0.8 g) and potassium carbonate (0.97 g) in acetonitrile (30 ml) under an atmosphere of argon. The reaction was heated at 80°C for 16 hours, then concentrated *in vacuo* and the residue partitioned between ethyl acetate and water. Combined organic extracts were washed with water, saturated aqueous sodium chloride solution and dried (MgSO₄). The solvent was removed *in vacuo* to give the product as a cream solid (1.05 g, 82%); NMR d (CD₃SOCD₃) 2.36 (s, 3H), 3.78 (s, 3H), 3.80 (s, 3H), 5.80 (s, 2H), 6.92 (dd, 1H), 7.21 (s, 1H), 7.27 (d, 1H), 7.36 (d, 1H), 7.48 (d, 1H), 7.52 (d, 1H).

15

Preparation 17

The procedure described in Preparation 16 above were repeated using the appropriate indole and benzyl halide. Thus were obtained the compounds described below.

20 Methyl 4-acetoxy-N-(3,4-dichlorobenzyl)indole-2-carboxylate

81% yield; NMR d (CD₃SOCD₃) 2.4 (s, 3H), 3.8 (s, 3H), 5.85 (s, 2H), 6.9 (m, 2H), 7.3 (m, 3H), 7.5 (m, 2H); *M/z* (+) 392.2 (MH⁺).

Methyl 4-acetoxy-N-(3,4-difluorobenzyl)indole-2-carboxylate

25 66% yield; NMR d (CD₃SOCD₃) 2.49 (s, 3H), 3.96 (s, 3H), 5.93 (s, 2H), 6.93 - 7.00 (m, 1H), 7.03 (d, 1H), 7.23 - 7.33 (m, 1H), 7.37 - 7.49 (m, 3H) 7.60 (d, 1H); *M/z* (+) 360 (MH⁺).

Methyl 4-acetoxy-N-benzylindole-2-carboxylate

78% yield; NMR d (CD₃SOCD₃) 2.38 (s, 3H), 3.81 (s, 3H), 5.85 (s, 2H), 6.89 (d, 1H), 7.05 (d, 2H), 7.17 - 7.34 (m, 5H), 7.48 (d, 1H).

Methyl 4-acetoxy-N-(3-chlorobenzyl)indole-2-carboxylate

88% yield; NMR d (CD₃SOCD₃) 2.28 (s, 3H), 3.74 (s, 3H), 5.77 (s, 2H), 6.80 - 6.90 (m, 2H), 7.07 (s, 1H), 7.16 - 7.27 (m, 4H), 7.38 (d, 1H); *M/z* (+) 358 (MH⁺).

5 Methyl 4-acetoxy-N-(4-chlorobenzyl)indole-2-carboxylate

27% yield; NMR d (CD₃SOCD₃) 2.37 (s, 3H), 3.81 (s, 3H), 5.82 (s, 2H), 6.90 (d, 1H), 7.08 (d, 2H), 7.31 (dd, 1H), 7.49 (d, 1H); *M/z* (+) 358 (MH⁺).

Preparation 18**10 Methyl N-(3,4-dichlorobenzyl)-4-hydroxy-5-methoxyindole-2-carboxylate**

Sodium methoxide (0.27 g) was added to a stirred solution of methyl 4-acetoxy-*N*-(3,4-dichlorobenzyl)-5-methoxyindole-2-carboxylate (1.05 g) in methanol (15 ml) under an atmosphere of argon. The reaction was stirred at room temperature for 2 hours, then concentrated *in vacuo* and the residue partitioned between ethyl acetate and water. Combined organic extracts were washed with water, saturated aqueous sodium chloride solution and dried (MgSO₄). The solvent was removed *in vacuo* and the residue purified by column chromatography using 45% diethyl ether : *iso*-hexane as eluent to give product as a white solid (0.83 g, 94%); NMR d (CD₃SOCD₃) 3.75 (s, 3H), 3.80 (s, 3H), 5.72 (s, 2H), 6.87 - 6.94 (m, 2H), 7.09 (d, 1H), 7.30 (s, 1H), 7.40 (s, 1H), 7.50 (d, 1H), 9.38 (s, 1H); *M/z* (+) 380 (MH⁺).

20

Preparation 19

The procedure described in Preparation 18 above was repeated using the appropriate acetoxyindole. Thus was obtained the compound described below.

25

Methyl N-(3,4-dichlorobenzyl)-4-hydroxyindole-2-carboxylate

97% yield; NMR d (CD₃SOCD₃) 3.8 (s, 3H), 5.65 (s, 2H), 6.5 (d, 1H), 6.9 (m, 2H), 7.1 (t, 1H), 7.3 (m, 1H), 7.45 (s, 1H), 7.5 (d, 1H), 9.9 (s, 1H); *M/z* (+) 350 (MH⁺).

30 **Methyl N-(3,4-difluorobenzyl)-4-hydroxyindole-2-carboxylate** 77% yield; NMR d (CD₃SOCD₃) 3.80 (s, 3H), 5.84 (s, 2H), 6.45 (d, 1H), 6.75 - 6.81 (m, 1H), 6.95 (d, 1H), 7.05 - 7.16 (m, 2H), 7.24 - 7.35 (m, 1H), 7.41 (s, 1H), 9.90 (s, 1H); *M/z* (+) 318 (MH⁺).

Methyl N-benzyl-4-hydroxyindole-2-carboxylate

90% yield; NMR d (CD₃SOCD₃) 3.80 (s, 3H), 5.77 (s, 2H), 6.43 (d, 1H), 6.90 - 7.28 (m, 7H), 7.40 (s, 1H), 9.88 (s, 1H); M/z (+) 282 (MH⁺).

5

Methyl N-(3-chlorobenzyl)-4-hydroxyindole-2-carboxylate

94% yield; NMR d (CD₃SOCD₃) 3.80 (s, 3H), 5.78 (s, 2H), 6.46 (d, 1H), 6.90 - 6.97 (m, 2H), 7.04 - 7.14 (m, 2H), 7.21 - 7.31 (m, 2H), 7.42 (s, 1H), 9.90 (s, 1H); M/z (+) 316 (MH⁺).

10 **Methyl N-(4-chlorobenzyl)-4-hydroxyindole-2-carboxylate**

77% yield; NMR d (CD₃SOCD₃) 3.80 (s, 3H), 5.75 (s, 2H), 6.45 (d, 1H), 6.94 (d, 1H), 7.00 (d, 2H), 7.10 (t, 1H), 7.30 (d, 2H), 7.40 (s, 1H), 9.89 (s, 1H); M/z (+) 316 (MH⁺).

Example 115 **Ethyl N-(3,4-dichlorobenzyl)-4-(3-hydroxypropylthio)indole-2-carboxylate (Ethyl ester of Compound 19)**

Sodium hydride (20 mg) was added to a stirred solution of ethyl N-(3,4-dichlorobenzyl)-4-mercaptoindole-2-carboxylate (170 mg) in DMF (7.5 ml). After 1 hour, 3-hydroxyprop-1-yl bromide (89 mg) was added and stirring continued for 16 hours. The 20 reaction mixture was partitioned between water and ethyl acetate and combined organic extracts were dried (MgSO₄), concentrated *in vacuo* and the residue purified by column chromatography using dichloromethane as eluent to give the product as a yellow gum (30 mg, 14%); M/z (+) 480.1 (MH⁺).

25 **Example 2****Ethyl N-(3,4-dichlorobenzyl)-4-(3-hydroxypropylsulphonyl)indole-2-carboxylate (Ethyl ester of Compound 20)**

Ethyl N-(3,4-dichlorobenzyl)-4-(3-hydroxypropylthio)indole-2-carboxylate (30 mg) was suspended in acetic acid (1 ml) and stirred in the presence of hydrogen peroxide (30%. 30 0.25 ml) for 16 hours. The reaction mixture was diluted with water (20 ml) and extracted with ethyl acetate. Combined organic extracts were dried (MgSO₄) and concentrated *in vacuo* to give the desired product as a colourless gum (30 mg, 93%); M/z (+) 512.4 (MH⁺).

Example 3**Methyl N-benzyl-4-(2-hydroxyethoxy)indole-2-carboxylate (Methyl ester of Compound 1)**

5 Potassium carbonate (0.2 g) was added to a stirred solution of methyl *N*-benzyl-4-hydroxyindole-2-carboxylate (0.2 g) and 2-Bromoethanol (98 mg) in DMF (15 ml). The reaction was then stirred for 16 hours at 80°C under an atmosphere of argon. The reaction mixture was concentrated *in vacuo* and the residue partitioned between ethyl acetate and water. Combined organic extracts were washed with water, dried (MgSO_4), concentrated *in vacuo* and the residue purified by column chromatography using 70% ethyl acetate : *iso*-hexane as eluent to give the product as a colourless crystalline solid. (64mg, 27%); NMR d (CD_3SOCD_3) 3.74 - 3.82 (m, 5H), 4.10 (m, 2H), 4.88 (m, 1H), 5.80 (s, 2H), 6.58 (m, 1H), 6.98 (d, 2H), 7.09 (m, 1H), 7.14 - 7.27 (m, 4H), 7.38 (m, 1H); M/z (+) 326 ($M\text{H}^+$).

10

15 Example 4

The procedure described in Example 3 above was repeated using the appropriate hydroxyindole and alkyl halide. Thus were obtained the compounds described below.

Methyl N-benzyl-4-(methoxyethoxy)indole-2-carboxylate (Methyl ester of Compound 2)

20 55% yield; NMR d (CD_3SOCD_3) 3.35 (s, 3H), 3.74 (m, 2H), 3.79 (s, 3H), 4.21 (m, 2H), 5.80 (s, 2H), 6.60 (d, 1H), 6.99 (d, 2H), 7.12 (d, 1H), 7.15 - 7.28 (m, 4H), 7.30 (s, 1H); M/z (+) 340 ($M\text{H}^+$).

Methyl N-(3,4-difluorobenzyl)-4-(methoxycarbonylmethoxy)indole-2-carboxylate (Di-**25 methyl ester of Compound 5)**

NMR d (CD_3SOCD_3) 3.72 (s, 3H), 3.82 (s, 3H), 4.94 (s, 2H), 5.80 (s, 2H), 6.54 (d, 1H), 6.79 (m, 1H), 7.10 - 7.36 (m, 5H); M/z (+) 390 ($M\text{H}^+$).

Methyl N-(3-chlorobenzyl)-4-(methoxycarbonylmethoxy)indole-2-carboxylate (Di-**30 methyl ester of Compound 6)**

93% yield; NMR d (CD_3SOCD_3) 3.72 (s, 3H), 3.82 (s, 3H), 4.93 (s, 2H), 5.81 (s, 2H), 6.54 (d, 1H), 6.93 (m, 1H), 7.08 (s, 1H), 7.14 - 7.37 (m, 6H); M/z (+) 388 ($M\text{H}^+$).

Methyl N-benzyl-4-(1-methoxycarbonyl-1-phenylmethoxy)indole-2-carboxylate (Di-methyl ester of Compound 7)

42% yield; NMR d (CD₃SOCD₃) 3.66 (s, 3H), 3.80 (s, 3H), 5.81 (s, 2H), 6.13 (s, 1H), 6.56 (dd, 1H), 7.00 (d, 2H), 7.14 - 7.29 (m, 5H), 7.37 - 7.50 (m, 4H), 7.62 (d, 2H); M/z (+) 430 (MH⁺).

Methyl N-(4-chlorobenzyl)-4-(methoxycarbonylmethoxy)indole-2-carboxylate (Di-methyl ester of Compound 8)

10 93% yield; NMR d (CD₃SOCD₃) 3.71 (s, 3H), 3.80 (s, 3H), 4.92 (s, 2H), 5.80 (s, 2H), 6.52 (d, 1H), 7.01 (d, 2H), 7.12 - 7.23 (m, 2H), 7.31 (m, 3H); M/z (+) 388 (MH⁺).

Methyl N-(3,4-dichlorobenzyl)-4-(2-(methoxycarbonyl)ethoxy)indole-2-carboxylate (Methyl Di-ester of Compound 12)

15 89% yield; NMR d (CD₃SOCD₃) 1.59 (d, 3H), 3.68 (s, 3H), 3.80 (s, 3H), 5.08 (q, 1H), 5.79 (s, 2H), 6.47 (d, 1H), 6.89 (dd, 1H), 7.14 - 7.24 (m, 2H), 7.33 - 7.36 (m, 2H), 7.51 (d, 1H); M/z (+) 436 (MH⁺).

Methyl N-(3,4-dichlorobenzyl)-4-[2-(2-methoxy-4-methoxycarbonylphenyl)ethoxy]-

20 **indole-2-carboxylate (Di-methyl ester of Compound 13)**

64% yield; NMR d (CD₃SOCD₃) 3.80 (s, 3H), 3.86 (s, 3H), 3.91 (s, 3H), 5.26 (s, 2H), 5.80 (s, 2H), 6.68 (d, 1H), 6.88 (dd, 1H), 7.16 (d, 1H), 7.25 (t, 1H), 7.33 (d, 1H), 7.40 (s, 1H), 7.50 (d, 1H), 7.54 (d, 1H), 7.59 - 7.69 (m, 2H); M/z (+) 528 (MH⁺).

25 **Methyl N-(3,4-dichlorobenzyl)-4-(2-hydroxyethoxy)indole-2-carboxylate (Methyl ester of Compound 14)**

63% yield; NMR d (CD₃SOCD₃) 3.75 - 3.81 (m, 5H), 4.10 (t, 2H), 4.88 (t, 1H), 5.80 (s, 2H), 6.60 (d, 1H), 6.88 (dd, 1H), 7.12 (d, 1H), 7.22 (t, 1H), 7.28 (d, 1H), 7.40 (s, 1H), 7.50 (d, 1H); M/z (+) 394 (MH⁺).

Methyl N-(3,4-dichlorobenzyl)-4-(3-hydroxypropoxy)indole-2-carboxylate (Methyl ester of Compound 15)

75% yield; NMR d (CD₃SOCD₃) 1.92 (s, 2H), 3.60 (dd, 2H), 4.17 (t, 2H), 4.53 (t, 1H), 5.79 (s, 2H), 6.61 (d, 1H), 6.87 (dd, 1H), 7.12 (d, 1H), 7.24 (t, 1H), 7.29 - 7.34 (m, 2H), 7.51 (d, 1H); M/z (+) 408 (MH⁺).

Methyl N-(3,4-dichlorobenzyl)-4-(dimethylaminoethoxy)indole-2-carboxylate (Methyl ester of Compound 18)

NMR d (CD₃SOCD₃) 2.26 (s, 6H), 2.71 (t, 2H), 3.80 (s, 3H), 4.18 (t, 2H), 5.79 (s, 2H), 6.61 (d, 1H), 6.87 (dd, 1H), 7.12 (d, 1H), 7.21 (d, 1H), 7.26 - 7.31 (m, 2H), 7.50 (d, 1H); M/z (+) 421 (MH⁺).

Methyl N-(3,4-dichlorobenzyl)-5-methoxy-4-(methoxycarbonylmethoxy)indole-2-carboxylate (Di-methyl ester of Compound 9)

15 87% yield; NMR d (CD₃SOCD₃) 3.67 (s, 3H), 3.79 (s, 3H), 3.82 (s, 3H), 4.83 (s, 2H), 5.79 (s, 2H), 6.90 (m, 1H), 7.16 - 7.30 (m, 2H), 7.36 (s, 2H), 7.52 (d, 1H); M/z (+) 452 (MH⁺).

Methyl N-(3,4-dichlorobenzyl)-5-methoxy-4-(3-morpholinopropoxy)indole-2-carboxylate (Methyl ester of Compound 10)

20 84% yield; NMR d (CD₃SOCD₃) 1.86 (m, 2H), 2.24 - 2.44 (m, 6H), 3.50 - 3.60 (m, 4H), 3.78 (s, 3H), 3.80 (s, 3H), 4.16 (t, 2H), 5.78 (s, 2H), 6.90 (d, 1H), 7.16 (d, 1H), 7.23 (d, 1H), 7.34 (m, 2H), 7.51 (d, 1H); M/z (+) 507 (MH⁺).

Methyl N-(3,4-dichlorobenzyl)-5-methoxy-4-carbamoylmethoxyindole-2-carboxylate

25 **(Methyl ester of Compound 11)**

74% yield; NMR d (CD₃SOCD₃) 3.80 (s, 3H), 3.81 (s, 3H), 4.52 (s, 2H), 5.77 (s, 2H), 6.89 (dd, 1H), 7.18 - 7.61 (m, 7H); M/z (+) 437 (MH⁺).

Methyl N-(3,4-dichlorobenzyl)-4-(2,3-epoxypropoxy)indole-2-carboxylate (precursor

30 **to 16, 17, 22 23, 24, 25, 26, 27 and 28)**

60% yield; NMR d (CD₃SOCD₃) 2.78 (m, 1H), 2.87 (m, 1H), 3.40 (m, 1H), 4.00 (dd, 1H), 4.46 (dd, 1H), 5.80 (s, 1H), 6.65 (d, 1H), 6.88 (dd, 1H), 7.16 (d, 1H), 7.24 (t, 1H), 7.31 (d, 1H), 7.36 (s, 1H), 7.50 (d, 1H); M/z (+) 406 (M⁺).

5 Example 5

N-Benzyl-4-(3-morpholinopropoxy)indole-2-carboxylic acid (Compound 3)

N-3-chloropropylmorpholine (128 mg) was added to a stirred solution of methyl N-(3,4-dichlorobenzyl)-4-hydroxyindole-2-carboxylate (0.2 g) and potassium carbonate (0.2 g) in DMF (3 ml). The reaction was then stirred for 48 hours at 80°C under an atmosphere of argon.

10 Water (4 ml) was added and the product was extracted with ethyl acetate. Combined organic extracts were washed with water and dried (MgSO₄), concentrated *in vacuo* and the residue purified by column chromatography using 10% methanol : dichloromethane as eluent to give the product as a light brown gum. The product was used without further purification.

15 Aqueous sodium hydroxide solution (2.0 M, 4 ml) was added to a stirred solution of the product in methanol (2 ml) and THF (4 ml). The reaction was stirred for 16 hours at room temperature. The solvent was removed *in vacuo* and the residue dissolved in water. Aqueous citric acid (1.0M) was added to the solution causing the precipitation of the product as a white solid. The solid was filtered and dried *in vacuo* (220 mg, 80%, 2 steps); NMR d (CD₃SOCD₃) 1.94 (m, 2H), 2.37 (m, 4H), 3.55 (m, 6H), 4.12 (t, 2H), 5.87 (s, 2H), 6.55 (d, 2H), 6.96 - 7.28 (m, 8H); M/z (-) 393 (M-H⁺).

Example 6

The procedure described in Example 5 above was repeated using the appropriate alkyl halide. Thus was obtained the compound described below.

25

N-Benzyl-4-(3-(4-methylpiperazin-1-yl)propoxy)indole-2-carboxylic acid (Compound 4)

72% yield (2 steps); NMR d (CD₃OD) 2.34 (m, 2H), 2.92 (s, 3H), 3.50 (t, 2H), 3.62 - 3.84 (d, 8H), 4.41 (t, 2H), 5.80 (s, 2H), 6.50 (d, 1H) 6.94 - 7.04 (m, 3H), 7.09 - 7.26 (m, 4H), 7.57 (s, 1H); M/z (-) 406 (M-H⁺).

30

Example 7

N-(3,4-Dichlorobenzyl)-4-(2-hydroxy-3-dimethylaminopropoxy)indole-2-carboxylic acid
(Compound 16)

Dimethylamine in methanol (2.0 M, 2.14 ml) was added to a stirred solution of methyl *N*-(3,4-dichlorobenzyl)-4-(2,3-epoxypropoxy)indole-2-carboxylate (87 mg) in DMF (5 ml) 5 under an atmosphere of argon. The reaction was heated at 80°C for 16 hours. The reaction was concentrated *in vacuo* and the residue partitioned between ethyl acetate and water. Combined organic extracts were washed with saturated aqueous sodium chloride solution, dried (MgSO_4) and concentrated *in vacuo*. The residue was dissolved in THF (3 ml) and methanol (1.5 ml) and NaOH (2M, 3 ml) added, and the reaction stirred for 16 hours. The 10 reaction was then concentrated *in vacuo* and the residue dissolved in water. The solution was acidified by dropwise addition of acetic acid, resulting in the precipitation of a white solid which was filtered, washed with water and dried *in vacuo* to give the desired end product as a white solid (50 mg, 54%, 2 steps); NMR d (CD_3SOCD_3) 2.85 (s, 6H), 3.22 - 3.40 (m, 2H), 4.01 - 4.14 (m, 1H), 5.81 (s, 2H), 6.01 (d, 1H), 6.60 (d, 1H), 6.88 (dd, 1H), 7.13 (d, 1H), 7.21 15 (t, 1H), 7.28 (d, 1H), 7.44 (s, 1H), 7.53 (d, 1H); M/z (-) 435 ($M\text{-H}^+$).

Example 8

The procedure described in Example 7 above was repeated using the appropriate amines. Thus were obtained the compounds described below.

20

N-(3,4-Dichlorobenzyl)-4-(2-hydroxy-3-morpholinopropoxy)indole-2-carboxylic acid
(Compound 17)

36% yield (2 steps); NMR d (CD_3SOCD_3) 2.83 - 3.41 (M, 6H), 3.65 - 3.84 (M, 4H), 4.07 (d, 2H), 4.31 (M, 1H), 5.80 (s, 2H), 6.60 (d, 1H), 6.89 (dd, 1H), 7.12 (d, 1H), 7.21 (t, 1H), 7.29 25 (d, 1H), 7.41 (s, 1H), 7.52 (d, 1H); M/z (+) 479 ($M\text{H}^+$).

Compound 23

13% yield (2 steps); M/z (-) 505.4 ($M\text{-H}^+$).

30 **Compound 24**

8% yield (2 steps); M/z (-) 520.3 ($M\text{-H}^+$).

Compound 25

30% yield (2 steps); M/z (-) 491.3 ($M-H^+$).

Compound 26

5 75% yield (2 steps); M/z (-) 523.4 ($M-H^+$).

Compound 27

66% yield (2 steps); M/z (-) 505.4 ($M-H^+$).

10 **Compound 28**

17% yield (2 steps); M/z (-) 465.2 ($M-H^+$).

Example 9

15 ***N*-(3,4-Dichlorobenzyl)-4-(2,3-dihydroxypropoxy)indole-2-carboxylic acid**

(Compound 21)

Ethyl *N*-(3,4-dichlorobenzyl)-4-(2,3-dihydroxypropoxy)indole-2-carboxylate (85 mg) was dissolved in THF (3 ml) and methanol (1 ml) and sodium hydroxide (2M, 3.0 ml) was added and the reaction stirred for 16 hours. The reaction was then concentrated *in vacuo* and 20 the residue dissolved in water. The solution was acidified by dropwise addition of acetic acid, resulting in the precipitation of a white solid which was filtered, washed with water and dried *in vacuo* to give the desired end product (70 mg, 81%); NMR d (CD_3SOCD_3) 3.40 - 3.53 (m, 3H), 3.98 - 4.10 (m, 2H), 5.80 (s, 2H), 6.59 (d, 1H), 6.88 (d, 1H), 7.09 (d, 1H), 7.20 (t, 1H), 7.30 (d, 1H), 7.36 (s, 1H), 7.51 (d, 1H); M/z (-) 408 ($M-H^+$).

25

Example 10

Methyl *N*-(3,4-dichlorobenzyl)-4-(2-(4-(2-hydroxyethyl)piperazin-1-yl)ethoxy)indole-2-carboxylate (Methyl ester of Compound 22)

Triphenylphosphine (0.55 g) was added in small portions over 2 hours to a stirred solution of 30 carbon tetrabromide (0.47 g) and methyl *N*-(3,4-dichlorobenzyl)-4-(hydroxyethoxy)indole-2-carboxylate (0.28 g) in dichloromethane (10 ml). The reaction was stirred for 3 hours at room temperature, concentrated *in vacuo* and the residue purified by column chromatography

using dichloromethane as eluent to give methyl *N*-(3,4-dichlorobenzyl)-4-(bromoethoxy)indole-2-carboxylate as a white solid (0.29 g, 90%); NMR d (CD₃SOCD₃) 3.80 (s, 3H), 3.89 (t, 2H), 4.46 (t, 2H), 5.80 (s, 2H), 6.62 (d, 1H), 6.88 (dd, 1H), 7.17 (d, 1H), 7.25 (t, 1H), 7.30 - 7.36 (m, 2H), 7.52 (d, 1H); *M/z* (+) 456, 458 (MH⁺).

5 1-(2-Hydroxyethyl)piperazine (175 mg) was added to a stirred solution of methyl *N*-(3,4-dichlorobenzyl)-4-(bromoethoxy)indole-2-carboxylate (278 mg) and potassium iodide (10 mg) in acetonitrile (15 ml). The reaction was heated at 80°C for 16 hours. The reaction was concentrated *in vacuo* and the residue dissolved in ethyl acetate, washed with water, saturated aqueous sodium chloride solution and dried (MgSO₄). The solvent was removed *in*
10 *vacuo* to give the product as a colourless oil (215 mg, 70%); NMR d (CD₃SOCD₃) 2.31 - 2.57 (m, 10H), 2.78 (t, 2H), 3.47 (dd, 2H), 3.80 (s, 3H), 4.20 (t, 2H), 4.30 (t, 1H), 5.79 (s, 2H), 6.61 (d, 1H), 6.88 (dd, 1H), 7.13 (d, 1H), 7.22 (t, 1H), 7.29 - 7.32 (m, 2H), 7.51 (d, 1H); *M/z* (+) 506 (MH⁺).

15 **Example 11**

***N*-(3,4-Dichlorobenzyl)-4-(3-hydroxypropoxy)indole-2-carboxylic acid (Compound 15)**

Methyl *N*-(3,4-Dichlorobenzyl)-4-(3-hydroxypropoxy)indole-2-carboxylate (6.9 g) was dissolved in THF (140 ml) and methanol (140 ml) and sodium hydroxide (3M, 110 ml) was added and the reaction stirred for 16 hours. The reaction was then concentrated *in vacuo* 20 and the residue dissolved in water. The solution was acidified by dropwise addition of acetic acid, resulting in the precipitation of a white solid which was filtered, washed with water and dried *in vacuo* to give the desired end product (5.58 g, 84%); NMR d (CD₃SOCD₃) 1.92 (m, 2H), 3.60 (dd, 2H), 4.13 (dd, 2H), 5.89 (s, 2H), 6.52 (s, 1H), 6.94 - 7.11 (m, 4H), 7.32 (d, 1H), 7.47 (d, 1H); *M/z* (-) 392 (M-H⁺).

25

Example 12

Using procedures similar to that described in Example 11 above but with the appropriate starting materials, the following compounds were prepared.

30 ***N*-(3,4-Dichlorobenzyl)-4-(3-hydroxypropylthio)indole-2-carboxylic acid (Compound 19)**
70% yield; *M/z* (-) 408.3 (M-H⁺).

N-(3,4-Dichlorobenzyl)-4-(3-hydroxypropylsulphonyl)indole-2-carboxylic acid**(Compound 20)**

41% yield; M/z (-) 440.3 ($M-H^+$).

5

N-Benzyl-4-(2-hydroxyethoxy)indole-2-carboxylic acid (Compound 1)

82% yield; NMR d (CD_3SOCD_3) 3.78 (d, 2H), 4.08 (t, 2H), 4.87 (m, 1H), 5.82 (s, 2H), 6.56 (d, 1H), 6.98 (d, 2H), 7.06 (d, 1H), 7.14 - 7.27 (m, 4H), 7.32 (s, 1H); M/z (-) 310 ($M-H^+$).

10 ***N-Benzyl-4-(2-methoxyethoxy)indole-2-carboxylic acid (Compound 2)***

91% yield; NMR d (CD_3SOCD_3) 3.35 (s, 3H), 3.74 (m, 2H), 4.20 (m, 2H), 5.81 (s, 2H), 6.57 (d, 1H), 6.99 (d, 2H), 7.08 (d, 1H), 7.14 - 7.27 (m, 5H), 12.84 (brs, 1H); M/z (-) 324 ($M-H^+$).

N-(3,4-Difluorobenzyl)-4-(carboxymethoxy)indole-2-carboxylic acid (Compound 5)

86% yield (2 steps); NMR d (CD_3SOCD_3) 4.79 (s, 2H), 5.80 (s, 2H), 6.48 (d, 1H), 6.78 (m,

15 1H), 7.08 - 7.36 (m, 5H), 12.98 (brs, 1H); M/z (-) 360 ($M-H^+$).***N-(3-Chlorobenzyl)-4-(carboxymethoxy)indole-2-carboxylic acid (Compound 6)***

89% yield; NMR d (CD_3SOCD_3) 4.79 (s, 2H), 5.81 (s, 2H), 6.45 (d, 1H), 6.91 (d, 1H), 7.05 - 7.31 (m, 6H); M/z (-) 358 ($M-H^+$).

20

N-Benzyl-4-(1-carboxy-1-phenylmethoxy)indole-2-carboxylic acid (Compound 7)

80% yield; NMR d (CD_3SOCD_3) 5.81 (s, 2H), 5.94 (s, 1H), 6.52 (d, 1H), 7.00 (d, 2H), 7.09 - 7.29 (m, 5H), 7.33 - 7.48 (m, 4H), 7.62 (d, 2H); M/z (-) 400 ($M-H^+$).

25 ***N-(4-Chlorobenzyl)-4-(carboxymethoxy)indole-2-carboxylic acid (Compound 8)***

66% yield; NMR d (CD_3SOCD_3) 4.79 (s, 2H), 5.81 (s, 2H), 6.48 (d, 1H), 7.00 (d, 2H), 7.06 - 7.20 (m, 2H), 7.26 - 7.34 (m, 3H), 12.95 (brs, 1H); M/z (-) 358 ($M-H^+$).

N-(3,4-Dichlorobenzyl)-4-(1-carboxyethoxy)indole-2-carboxylic acid (Compound 12)

30 90% yield; NMR d (CD_3SOCD_3) 1.58 (d, 3H), 4.91 (q, 1H), 5.80 (s, 2H), 6.42 (d, 1H), 6.88 (dd, 1H), 7.08 - 7.21 (m, 2H), 7.29 (s, 1H), 7.31 (d, 1H), 7.50 (d, 1H); M/z (-) 406 ($M-H^+$).

N-(3,4-Dichlorobenzyl)-4-(2-(4-carboxy-2-methoxyphenyl)ethoxy)indole-2-carboxylic acid (Compound 13)

81% yield; NMR d (CD₃SOCD₃) 3.91 (s, 3H), 5.26 (s, 2H), 5.81 (s, 2H), 6.66 (d, 1H), 6.90 (dd, 1H), 7.10 - 7.25 (m, 2H), 7.32 - 7.36 (m, 2H), 7.48 - 7.56 (m, 2H), 7.59 - 7.65 (m, 2H), 5 12.97 (brs, 1H); *M/z* (-) 498 (*M-H*⁺).

N-(3,4-Dichlorobenzyl)-4-(2-hydroxyethoxy)indole-2-carboxylic acid (Compound 14)

83% yield; NMR d (CD₃SOCD₃) 3.77 (m, 2H), 4.08 (t, 2H), 4.89 (m, 1H), 5.80 (s, 2H), 6.58 (d, 1H), 6.89 (dd, 1H), 7.08 (d, 1H), 7.19 (t, 1H), 7.29 (d, 1H), 7.34 (s, 1H), 7.50 (d, 1H); *M/z* 10 (-) 378 (*M-H*⁺).

N-(3,4-Dichlorobenzyl)-4-(dimethylaminoethoxy)indole-2-carboxylic acid (Compound 18)

13% yield (2 steps); NMR d (CD₃SOCD₃) 2.89 (s, 6H), 3.59 (t, 2H), 4.45 (t, 2H), 5.81 (s, 2H), 15 6.64 (d, 1H), 6.90 (dd, 1H), 7.18 (d, 1H), 7.22 (d, 1H), 7.28 (m, 1H), 7.48 - 7.54 (m, 2H); *M/z* (-) 405 (*M-H*⁺).

N-(3,4-Dichlorobenzyl)-4-(2-(4-(2-hydroxyethyl)piperazin-1-yl)ethoxy)indole-2-carboxylic acid (Compound 22)

20 90% yield; NMR d (CD₃SOCD₃) 2.40 - 2.67 (m, 10H), 2.81 (t, 2H), 3.49 (dd, 2H), 4.20 (t, 2H), 5.82 (s, 2H), 6.59 (d, 1H), 6.92 (dd, 1H), 7.06 (d, 1H), 7.11 - 7.19 (m, 2H), 7.30 (s, 1H), 7.50 (d, 1H); *M/z* (-) 490 (*M-H*⁺).

N-(3,4-Dichlorobenzyl)-5-methoxy-4-(carboxymethoxy)indole-2-carboxylic acid

25 **(Compound 9)**

91% yield; NMR d (CD₃SOCD₃) 3.77 (s, 3H), 4.64 (s, 2H), 5.78 (s, 2H), 6.91 (dd, 1H), 7.13 (d, 1H), 7.20 (d, 1H), 7.30 (s, 1H), 7.35 (d, 1H), 7.51 (d, 1H); *M/z* (+) 424 (*MH*⁺).

N-(3,4-Dichlorobenzyl)-5-methoxy-4-(3-morpholinopropoxy)indole-2-carboxylic acid

30 **(Compound 10)**

94% yield; NMR d (CD₃SOCD₃) 2.17 (m, 2H), 3.10 (m, 2H), 3.34 (m, 4H), 3.79 (t, 2H), 3.80 (s, 3H), 3.97 (m, 2H), 4.20 (t, 2H), 5.79 (s, 2H), 6.93 (dd, 1H), 7.16 (d, 1H), 7.25 (d, 1H), 7.31 (s, 1H), 7.34 (d, 1H), 7.52 (d, 1H); *M/z* (+) 493 (*MH*⁺).

5 **Example 13**

N-(3,4-Dichlorobenzyl)-5-methoxy-4-(carbamoylmethoxy)indole-2-carboxylic acid (Compound 11)

Lithium iodide (0.39 g) was added to stirred solution of methyl *N*-(3,4-dichlorobenzyl)-5-methoxy-4-carbamoylmethoxyindole-2-carboxylate (0.12 g) in pyridine (10 ml) under an atmosphere of argon. The reaction was then heated at 115°C for 16 hours. The solvent was removed *in vacuo* and the residue was partitioned between ethyl acetate and hydrochloric acid (2.0 M, 10 ml). Combined organic extracts were washed with water and saturated aqueous sodium chloride solution, dried (MgSO₄) and concentrated *in vacuo* to give the product as a cream coloured solid (35 mg, 30%); NMR d (CD₃SOCD₃) 3.80 (s, 3H), 4.51 (s, 2H), 5.79 (s, 2H), 6.90 (dd, 1H), 7.18 (d, 1H), 7.25 (d, 1H), 7.34 (d, 1H), 7.39 (s, 1H), 7.42 (brs, 1H), 7.51 (d, 1H), 7.57 (brs, 1H), 13.05 (brs, 1H); *M/z* (-) 421 (*M-H*⁺).

Example 14

Biological Testing.

20 The following biological test methods, data and Examples serve to illustrate the present invention.

Abbreviations:

ATCC	American Type Culture Collection, Rockville, USA.
BCA	Bicinchoninic acid, (used, with copper sulphate, to assay protein)
BSA	Bovine Serum Albumin
DMEM	Dulbecco's modified Eagle's medium
EGTA	Ethylenebis(oxyethylenenitrilo)tetraacetic acid
FCS	Foetal calf serum
HEPES	(N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulphonic acid])
HBSS	Hank's Balanced Salt Solution
hMCP-1	Human Monocyte Chemoattractant Protein-1
PBS	Phosphate buffered saline

PCR Polymerase chain reaction

AMPLITAQ™, available from Perkin-Elmer Cetus, is used as the source of thermostable DNA polymerase.

Binding Buffer is 50 mM HEPES, 1 mM CaCl₂, 5 mM MgCl₂, 0.5% foetal calf serum, adjusted to pH 7.2 with 1 M NaOH.

5 Non-Essential Amino Acids (100X concentrate) is: L-Alanine, 890 mg/l; L-Asparagine, 1320 mg/l; L-Aspartic acid, 1330 mg/l; L-Glutamic acid, 1470 mg/l; Glycine, 750 mg/l; L-Proline, 1150 mg/l and; L-Serine, 1050 mg/l.

Hypoxanthine and Thymidine Supplement (50x concentrate) is: hypoxanthine, 680 mg/l and; thymidine, 194 mg/l.

10 Penicillin-Streptomycin is: Penicillin G (sodium salt); 5000 units/ml; Streptomycin sulphate, 5000 µg/ml.

Human monocytic cell line THP-1 cells are available from ATCC, accession number ATCC TIB-202.

15 Hank's Balanced Salt Solution (HBSS) was obtained from Gibco; see *Proc. Soc. Exp. Biol. Med.*, 1949, **71**, 196.

Synthetic cell culture medium, RPMI 1640 was obtained from Gibco; it contains inorganic salts [Ca(NO₃)₂.4H₂O 100 mg/l; KCl 400 mg/l; MgSO₄.7H₂O 100 mg/l; NaCl 6000 mg/l; NaHCO₃ 2000 mg/l & Na₂HPO₄ (anhyd) 800 mg/l], D-Glucose 2000 mg/l, reduced glutathione 1 mg/l, amino acids and vitamins.

20 FURA-2/AM is 1-[2-(5-carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxy]-2-(2'-amino-5'-methylphenoxy)-ethane-*N,N,N',N'*-tetraacetic acid pentaacetoxymethyl ester and was obtained from Molecular Probes, Eugene, Oregon, USA.

Blood Sedimentation Buffer contains 8.5g/l NaCl and 10g/l hydroxyethyl cellulose.

Lysis Buffer is 0.15M NH₄Cl, 10mM KHCO₃, 1mM EDTA

25 Whole Cell Binding Buffer is 50 mM HEPES, 1 mM CaCl₂, 5 mM MgCl₂, 0.5% BSA, 0.01% NaN₃, adjusted to pH 7.2 with 1M NaOH.

Wash buffer is 50mM HEPES. 1mM CaCl₂, 5mM MgCl₂, 0.5% heat inactivated FCS, 0.5MNaCl adjusted to pH7.2 with 1M NaOH.

30 General molecular biology procedures can be followed from any of the methods described in "Molecular Cloning - A Laboratory Manual" Second Edition, Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory, 1989).

i) Cloning and expression of hMCP-1 receptor

The MCP-1 receptor B (CCR2B) cDNA was cloned by PCR from THP-1 cell RNA using suitable oligonucleotide primers based on the published MCP-1 receptor sequences (Charo *et al.*, 1994, *Proc. Natl. Acad. Sci. USA*, **91**, 2752). The resulting PCR products were 5 cloned into vector PCR-II™ (InVitrogen, San Diego, CA.). Error free CCR2B cDNA was subcloned as a Hind III-Not I fragment into the eukaryotic expression vector pCDNA3 (InVitrogen) to generate pCDNA3/CC-CCR2A and pCDNA3/CCR2B respectively.

Linearised pCDNA3/CCR2B DNA was transfected into CHO-K1 cells by calcium phosphate precipitation (Wigler *et al.*, 1979, *Cell*, **16**, 777). Transfected cells were selected by 10 the addition of Geneticin Sulphate (G418, Gibco BRL) at 1mg/ml, 24 hours after the cells had been transfected. Preparation of RNA and Northern blotting were carried out as described previously (Needham *et al.*, 1995, *Prot. Express. Purific.*, **6**, 134). CHO-K1 clone 7 (CHO-CCR2B) was identified as the highest MCP-1 receptor B expressor.

ii) Preparation of membrane fragments

15 CHO-CCR2B cells were grown in DMEM supplemented with 10% foetal calf serum, 2 mM glutamine, 1x Non-Essential Amino Acids, 1x Hypoxanthine and Thymidine Supplement and Penicillin-Streptomycin (at 50 µg streptomycin/ml, Gibco BRL). Membrane fragments were prepared using cell lysis/differential centrifugation methods as described previously (Siciliano *et al.*, 1990, *J. Biol. Chem.*, **265**, 19658). Protein concentration was 20 estimated by BCA protein assay (Pierce, Rockford, Illinois) according to the manufacturer's instructions.

iii) Assay

¹²⁵I MCP-1 was prepared using Bolton and Hunter conjugation (Bolton *et al.*, 1973, *Biochem. J.*, **133**, 529; Amersham International plc]. Equilibrium binding assays were carried 25 out using the method of Ernst *et al.*, 1994, *J. Immunol.*, **152**, 3541. Briefly, varying amounts of ¹²⁵I-labeled MCP-1 were added to 7µg of purified CHO-CCR2B cell membranes in 100 µl of Binding Buffer. After 1 hour incubation at room temperature the binding reaction mixtures were filtered and washed 5 times through a plate washer (Brandel MLR-96T Cell Harvester) using ice cold Binding Buffer. Filter mats (Brandel GF/B) were pre-soaked for 60 minutes in 30 0.3% polyethylenimine prior to use. Following filtration individual filters were separated into 3.5ml tubes (Sarstedt No. 55.484) and bound ¹²⁵I-labeled MCP-1 was determined (LKB 1277 Gammamaster). Cold competition studies were performed as above using 100 pM ¹²⁵I-labeled

MCP-1 in the presence of varying concentrations of unlabelled MCP-1. Non-specific binding was determined by the inclusion of a 200-fold molar excess of unlabelled MCP-1 in the reaction.

Ligand binding studies with membrane fragments prepared from CHO-CCR2B cells 5 showed that the CCR2B receptor was present at a concentration of 0.2 pmoles/mg of membrane protein and bound MCP-1 selectively and with high affinity ($IC_{50} = 110$ pM, $K_d = 120$ pM). Binding to these membranes was completely reversible and reached equilibrium after 45 minutes at room temperature, and there was a linear relationship between MCP-1 binding and CHO-CCR2B cell membrane concentration when using MCP-1 at concentrations 10 between 100 pM and 500 pM.

Test compounds dissolved in DMSO (5 μ l) were tested in competition with 100 pM labelled MCP-1 over a concentration range (0.01-50 μ M) in duplicate using eight point dose-response curves and IC_{50} concentrations were calculated.

Compounds tested of the present invention had IC_{50} values of 50 μ M or less in the 15 hMCP-1 receptor binding assay described herein. For example, compound 5 in Table 1 had an IC_{50} of 2.3 μ M.

b) MCP-1 mediated calcium flux in THP-1 cells

The human monocytic cell line THP-1 was grown in a synthetic cell culture medium RPMI 1640 supplemented with 10 % foetal calf serum, 6mM glutamine and 20 Penicillin-Streptomycin (at 50 μ g streptomycin/ml, Gibco BRL). THP-1 cells were washed in HBSS (lacking Ca^{2+} and Mg^{2+}) + 1 mg/ml BSA and resuspended in the same buffer at a density of 3×10^6 cells/ml. The cells were then loaded with 1mM FURA-2/AM for 30 min at 37°C, washed twice in HBSS, and resuspended at 1×10^6 cells/ml. THP-1 cell suspension (0.9 ml) was added to a 5 ml disposable cuvette containing a magnetic stirrer bar and 2.1 ml of 25 prewarmed (37°C) HBSS containing 1 mg/ml BSA, 1 mM $MgCl_2$ and 2 mM $CaCl_2$. The cuvette was placed in a fluorescence spectrophotometer (Perkin Elmer, Norwalk, CT) and preincubated for 4 min at 37°C with stirring. Fluorescence was recorded over 70 sec and cells were stimulated by addition of hMCP-1 to the cuvette after 10 sec. $[Ca^{2+}]_i$ was measured by excitation at 340 nm and 380 nm alternately and subsequent measurement of the intensity of 30 the fluorescence emission at 510 nm. The ratio of the intensities of the emitted fluorescent light following excitation at 340 nm and 380 nm, (R), was calculated and displayed to give and estimate of cytoplasmic $[Ca^{2+}]$ according to the equation:-

$$[\text{Ca}^{2+}]_i = K_d \frac{(R - R_{\min})}{(R_{\max} - R)}$$

where the K_d for FURA-2 Ca^{2+} complex at 37°C was taken to be 224nm. R_{\max} is the maximal fluorescence ratio determined after addition of 10 mM Ionomycin, R_{\min} is the minimal ratio determined by the subsequent addition of a Ca^{2+} free solution containing 5 mM EGTA, and Sf2/Sb2 is the ratio of fluorescence values at 380 nm excitation determined at R_{\min} and R_{\max} , respectively.

Stimulation of THP-1 cells with hMCP-1 induced a rapid, transient rise in $[\text{Ca}^{2+}]_i$ in a specific and dose dependent manner. Dose response curves indicated an approximate EC_{50} of 2 nm. Test compounds dissolved in DMSO (10 μ l) were assayed for inhibition of calcium release by adding them to the cell suspension 10 sec prior to ligand addition and measuring the reduction in the transient rise in $[\text{Ca}^{2+}]_i$. Test compounds were also checked for lack of agonist activity by addition in place of hMCP-1.

c) hMCP-1 and RANTES mediated chemotaxis.

15 *In vitro* chemotaxis assays were performed using the human monocytic cell line THP-1. Cell migration through polycarbonate membranes was measured by enumerating those passing through either directly by Coulter counting or indirectly by use of a colourimetric viability assay measuring the cleavage of a tetrazolium salt by the mitochondrial respiratory chain (Scudiero D.A. *et al.* 1988, *Cancer Res.*, **48**, 4827-4833).

20 Chemoattractants were introduced into a 96-well microtitre plate which forms the lower well of a chemotaxis chamber fitted with a PVP-free 5 μ m poresize polycarbonate adhesive framed filter membrane (NeuroProbe MB series, Cabin John, MD 20818, USA) according to the manufacturer's instructions. The chemoattractant was diluted as appropriate in synthetic cell culture medium, RPMI 1640 (Gibco) or supplemented with 2 mM glutamine and 0.5% BSA, or alternatively with HBSS with Ca^{2+} and Mg^{2+} without Phenol Red (Gibco) plus 0.1% BSA. Each dilution was degassed under vacuum for 30 min and was placed (400 μ l) in the lower wells of the chamber and THP-1 cells (5×10^5 in 100 μ l RPMI 1640 + 0.5% BSA) were incubated in each well of the upper chamber. For the inhibition of chemotaxis the chemoattractant was kept at a constant submaximal concentration determined previously (1nM MCP-1) and added to the lower well together with the test compounds dissolved in DMSO (final DMSO concentration < 0.05% v/v) at varying concentrations. The chamber was incubated for 2 h at 37°C under 5 % CO_2 . The medium was removed from the

upper wells which were then washed out with 200 µl physiological saline before opening the chamber, wiping dry the membrane surface and centrifuging the 96-well plate at 600 g for 5 min to harvest the cells. Supernatant (150 µl) was aspirated and 10 µl of cell proliferation reagent, WST-1, {4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-phenyl disulfonate} plus an electron coupling reagent (Boehringer Mannheim, Cat.no. 1644 807) was added back to the wells. The plate was incubated at 37°C for 3 h and the absorbance of the soluble formazan product was read on a microtitre plate reader at 450 nm. The data was input into a spreadsheet, corrected for any random migration in the absence of chemoattractant and the average absorbance values, standard error of the mean, and significance tests were calculated. hMCP-1 induced concentration dependent cell migration with a characteristic biphasic response, maximal 0.5-1.0 nm.

In an alternative form of the above assay, fluorescently tagged cells can be used in order to assist in end point detection. In this case, the THP-1 cells used are fluorescently tagged by incubation in the presence of 5mM Calcein AM (Glycine, N,N'-[[3',6'-bis(acetyloxy)-3-oxospiro[isobenzofuran-1(3H),9'-[9H]xanthene]-2',7'-diyl]bis(methylene)]bis[N-[2-[(acetyloxy)methoxy]-2-oxoethyl]]-bis[(acetyloxy)methyl] ester; Molecular Probes) for 45 minutes in the dark. Cells are harvested by centrifugation and resuspended in HBSS (without Phenol Red) with Ca²⁺, Mg²⁺ and 0.1% BSA. 50µl (2x10⁵ cells) of the cell suspension are placed on the filter above each well and, as above, the unit is incubated at 37°C for 2 hours under 5% CO₂. At the end of the incubation, cells are washed off the upper face of the filter with phosphate buffered saline, the filter removed from the plate and the number of cells attracted to either the underside of the filter or the lower well estimated by reading fluorescence at 485nm excitation, 538nm emission wavelengths (fmax, Molecular Devices). The data was input into a spreadsheet, corrected for any random migration in the absence of chemoattractant and the average fluorescence values, standard error of the mean, percentage inhibition and IC₅₀ of compounds under test and significance tests can be calculated. In addition to MCP-1 induced chemotaxis, this alternative form of the assay was also used to measure inhibition of RANTES (2nM) induced chemotaxis.

d) Binding to human peripheral blood mononuclear cells(PBMCs)

30 **i) Preparation of human PBMCs**

Fresh human blood (200ml) was obtained from volunteer donors, collected into sodium citrate anticoagulant to give a final concentration of 0.38%. The blood was mixed

with Sedimentation Buffer and incubated at 37°C for 20 minutes. The supernatant was collected and centrifuged at 1700rpm for 5 minutes (Sorvall RT6000D). The pellet obtained was resuspended in 20 ml RPMI/BSA (1mg/ml) and 4 x 5mls of cells were carefully layered over 4 x 5mls of Lymphoprepä (Nycomed) in 15ml centrifuge tubes. Tubes were spun at 5 1700rpm for 30 minutes (Sorvall RT6000D) and the resultant layer of cells was removed and transferred to 50ml Falcon tubes. The cells were washed twice in Lysis Buffer to remove any remaining red blood cells followed by 2 washes in RPMI/BSA. Cells were resuspended in 5mls of Binding Buffer. Cell number was measured on a Coulter counter and additional binding buffer was added to give a final concentration of 1.25×10^7 PBMCs /ml.

10 ii) Assay

[^{125}I]MCP-1 was prepared using Bolton and Hunter conjugation (Bolton *et al.* 1973, *Biochem. J.*, **133**, 529; Amersham International plc]. Equilibrium binding assays were carried out using the method of Ernst *et al.*, 1994, *J. Immunol.*, **152**, 3541. Briefly, 50 μl of ^{125}I -labeled MCP-1 (final concentration 100pM) was added to 40 μl (5×10^5 cells) of cell suspension in a 96 15 well plate. Compounds, diluted in Whole Cell Binding Buffer from a stock solution of 10mM in DMSO were added in a final volume of 5 μl to maintain a constant DMSO concentration in the assay of 5%. Total binding was determined in the absence of compound. Non-specific binding was defined by the addition of 5 μl cold MCP-1 to give a final assay concentration of 100nM. Assay wells were made up to a final volume of 100 μl with Whole Cell Binding 20 Buffer and the plates sealed. Following incubation at 37°C for 60 minutes the binding reaction mixtures were filtered and washed for 10 seconds using ice cold Wash Buffer using a plate washer (Brandel MLR-96T Cell Harvester). Filter mats (Brandel GF/B) were pre-soaked for 60 minutes in 0.3% polyethylenimine plus 0.2% BSA prior to use. Following filtration individual filters were separated into 3.5ml tubes (Sarstedt No. 55.484) and bound ^{125}I -labeled 25 MCP-1 was determined (LKB 1277 Gammamaster).

Test compound potency was determined by assay in duplicate using six point dose-response curves and IC_{50} concentrations were determined.

For example, using this method, compound No. 9 in Table I showed an IC_{50} of 12.75 μM in the hMCP-1 chemotaxis assay and compound No. 15 in Table 1 showed an IC_{50} 30 of 3.64 μM in the RANTES chemotaxis assay.

No physiologically unacceptable toxicity was observed at the effective dose for compounds tested of the present invention.

Example 15**Pharmaceutical Compositions**

The following Example illustrates, but is not intended to limit, pharmaceutical dosage forms of the invention as defined herein (the active ingredient being termed "Compound X"), for therapeutic or prophylactic use in humans:

5 (a)

<u>Tablet I</u>	<u>mg/tablet</u>
Compound X.	100
Lactose Ph.Eur	182.75
Croscarmellose sodium	12.0
Maize starch paste (5% w/v paste)	2.25
Magnesium stearate	3.0

10 (b)

<u>Tablet II</u>	<u>mg/tablet</u>
Compound X	50
Lactose Ph.Eur	223.75
Croscarmellose sodium	6.0
Maize starch	15.0
Polyvinylpyrrolidone (5% w/v paste)	2.25
Magnesium stearate	3.0

(c)

<u>Tablet III</u>	<u>mg/tablet</u>
Compound X	1.0
Lactose Ph.Eur	93.25
Croscarmellose sodium	4.0
Maize starch paste (5% w/v paste)	0.75
Magnesium stearate	1.0

(d)

<u>Capsule</u>	<u>mg/capsule</u>
Compound X	10
Lactose Ph.Eur	488.5
Magnesium	1.5

(e)

<u>Injection I</u>	<u>(50 mg/ml)</u>
Compound X	5.0% w/v
1M Sodium hydroxide solution	15.0% v/v
0.1M Hydrochloric acid	to adjust pH to 7.6
Polyethylene glycol 400	4.5% w/v
Water for injection	to 100%

5

(f)

<u>Injection II</u>	<u>(10 mg/ml)</u>
Compound X	1.0% w/v
Sodium phosphate BP	3.6% w/v
0.1M Sodium hydroxide solution	15.0% v/v
Water for injection	to 100%

(g)

<u>Injection III</u>	<u>(1mg/ml, buffered to pH6)</u>
Compound X	0.1% w/v
Sodium phosphate BP	2.26% w/v
Citric acid	0.38% w/v
Polyethylene glycol 400	3.5% w/v
Water for injection	to 100%

(h)

<u>Aerosol I</u>	<u>mg/ml</u>
Compound X	10.0
Sorbitan trioleate	13.5
Trichlorofluoromethane	910.0
Dichlorodifluoromethane	490.0

(i)

<u>Aerosol II</u>	<u>mg/ml</u>
Compound X	0.2
Sorbitan trioleate	0.27
Trichlorofluoromethane	70.0
Dichlorodifluoromethane	280.0
Dichlorotetrafluoroethane	1094.0

5 (j)

<u>Aerosol III</u>	<u>mg/ml</u>
Compound X	2.5
Sorbitan trioleate	3.38
Trichlorofluoromethane	67.5
Dichlorodifluoromethane	1086.0
Dichlorotetrafluoroethane	191.6

(k)

<u>Aerosol IV</u>	<u>mg/ml</u>
Compound X	2.5
Soya lecithin	2.7
Trichlorofluoromethane	67.5
Dichlorodifluoromethane	1086.0
Dichlorotetrafluoroethane	191.6

(l)

<u>Ointment</u>	<u>ml</u>
Compound X	40 mg
Ethanol	300 μ l
Water	300 μ l
1-Dodecylazacycloheptan-2-one	50 μ l
Propylene glycol	to 1 ml

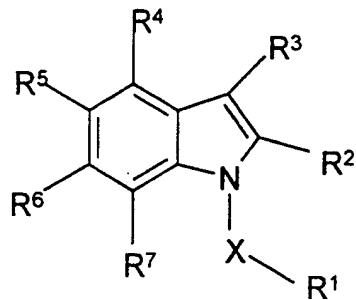
Note:

Compound X in the above formulation may comprise a compound illustrated in 5 Examples herein. The above formulations may be obtained by conventional procedures well known in the pharmaceutical art. The tablets (a)-(c) may be enteric coated by conventional means, for example to provide a coating of cellulose acetate phthalate. The aerosol formulations (h)-(k) may be used in conjunction with standard, metered dose aerosol dispensers, and the suspending agents sorbitan trioleate and soya lecithin may be replaced by 10 an alternative suspending agent such as sorbitan monooleate, sorbitan sesquioleate, polysorbate 80, polyglycerol oleate or oleic acid.

Claims

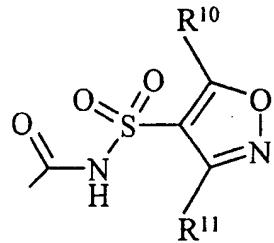
1. A compound of formula (I)

5



(I)

or a pharmaceutically acceptable salt, *in vivo* hydrolysable ester, or amide thereof, where
 10 X is CH_2 or SO_2 ;
 R^1 is an optionally substituted aryl or heteroaryl ring;
 R^2 is carboxy, cyano, $-\text{C}(\text{O})\text{CH}_2\text{OH}$, $-\text{CONHR}^8$, $-\text{SO}_2\text{NHR}^9$, tetrazol-5-yl, SO_3H , or a group of
 formula (VI)



15 (VI)

where R^8 is selected from hydrogen, alkyl, aryl, cyano, hydroxy, $-\text{SO}_2\text{R}^{12}$ where R^{12} is alkyl, aryl, heteroaryl, or haloalkyl, or R^8 is a group- $(\text{CHR}^{13})_r\text{-COOH}$ where r is an integer of 1-3 and each R^{13} group is independently selected from hydrogen or alkyl; R^9 is hydrogen, alkyl, optionally substituted aryl, optionally substituted heteroaryl, or a group COR^{14} where R^{14} is
 20 alkyl, aryl, heteroaryl or haloalkyl; R^{10} and R^{11} are independently selected from hydrogen or alkyl;
 R^3 is hydrogen, a functional group, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted heterocyclyl,

optionally substituted alkoxy, optionally substituted aralkyl, optionally substituted aralkyloxy, or optionally substituted cycloalkyl;

R⁴ is a group OR¹⁵ or S(O)_qR¹⁵, where q is 0, 1 or 2 and R¹⁵ is a substituted hydrogen-containing alkyl group; and

5 R⁵, R⁶ and R⁷ are independently selected from hydrogen, a functional group or an optionally substituted hydrocarbyl group or optionally substituted heterocyclic group.

2. A compound according to claim 1 wherein R¹⁵ is a C₁₋₃alkyl group substituted with one or more of a functional group, aryl optionally substituted by a functional group or a

10 heterocyclyl group optionally substituted by a functional group.

3. A compound according to claim 1 or claim 2 wherein R¹⁵ is a C₁₋₃alkyl group substituted with one or more groups selected from halo; hydroxy; cyano; amino, mono- or di-alkylamino wherein each alkyl group is optionally substituted by hydroxy, alkoxy or

15 heterocyclyl; C₁₋₄ alkoxy; carboxy; sulphonamido; CONH₂; morpholino; tetrahydropyrazinyl which is optionally N-substituted by alkyl or hydroxyalkyl; tetrahydropyridyl optionally substituted by hydroxy or hydroxyalkyl, pyridyl, pyrimidinyl, phenyl optionally substituted by carboxy, halo, hydroxy, alkoxy, carbamoyl, acyl or hydroxyalkyl where the alkyl group suitably includes at least two carbon atoms.

20

4. A compound according to any one of the preceding claims where R⁴ is a group OR¹⁵ where R¹⁵ is a straight or branched chain alkyl group which carries at least one hydroxy group.

25 5. A compound according to any one of the preceding claims wherein R¹⁵ is a group of formula -(CH₂)_a[(CHOH)(CH₂)_b]_dCH₂OH where a is an integer of from 1 to 4, b is 0 or an integer of from 1 to 4, and d is 0 or 1.

6. A compound according to any one of the preceding claims wherein R¹ is 3,4-dichlorophenyl, 3-fluoro-4-chlorophenyl, 3-chloro-4-fluorophenyl or 2,3-dichloropyrid-5-yl.

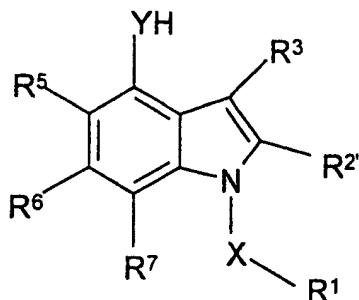
7. A compound according to any one of the preceding claims wherein X is CH₂.

8. A pharmaceutical composition comprising a compound according to any one of the preceding claims in combination with a pharmaceutically acceptable carrier.

5 9. A compound according to any one of claims 1 to 7 for use in the preparation of a medicament for use in the treatment of inflammatory disease.

10. A method of making a compound of formula (I) as defined in claim 1 which method comprises reacting a compound of formula (VII)

10



(VII)

where R¹, R³, R⁵, R⁶, R⁷ and X are as defined in relation to formula (I), R^{2'} is a group R² as defined in relation to formula (I) or a protected form thereof and Y is oxygen or sulphur with
15 a compound of formula (VIII)



(VIII)

20 where Z is a leaving group and R^{15'} is a group R¹⁵ as defined in claim 1 or a precursor thereof, and thereafter if desired or necessary carrying out one or more of the following steps:

- (i) converting a precursor group R^{15'} to a group R¹⁵;
- (ii) converting a group R¹⁵ to another such group;
- (iii) oxidising a thiol group R⁴ to a sulphinyl or sulphonyl group;
- 25 (iv) deprotecting a group R^{2'} or converting the existing group R² to a different R² group.

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/GB 00/00275

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D209/42 C07D401/12 C07D405/12 A61K31/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 288 743 A (BROOKS ET. AL.) 22 February 1994 (1994-02-22) column 2, line 16 - line 21; claims; examples ---	1-10
A	WO 93 12780 A (MERRELL DOW) 8 July 1993 (1993-07-08) claims; examples ---	1-10
P, X	WO 99 33800 A (HOECHST) 8 July 1999 (1999-07-08) claims; examples ---	1, 7, 8
P, Y	WO 99 07351 A (ZENECA) 18 February 1999 (1999-02-18) cited in the application page 18, line 17 - line 19; claims; examples ---	1-10 -/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

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T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search

11 May 2000

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 00/00275

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,Y	WO 99 07678 A (ZENECA) 18 February 1999 (1999-02-18) cited in the application page 17, line 8 - line 17; claims; examples -----	1-10

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 00/00275

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